

THE SIGNIFICANCE OF THE CONCENTRIC CORPUSCLES OF HASSALL

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SEVEN PLATES (FORTY-FIVE FIGURES)

INTRODUCTION

Notwithstanding much histologic study of the concentric corpuscles (Ecker, '53) of the mammalian thymus, there is as yet no agreement regarding the correct interpretation of their significance. Only recently has the problem been attacked by the experimental methods of autoplasmic transplants and tissue cultures. These methods have demonstrated that the earlier and more common interpretation of the corpuscles of Hassall in terms of atrophic remnants of the original tubular entodermal primordia lacks adequate support. Transplants of a complete lobe of fully differentiated thymus bodies of young guinea-pigs into the abdominal wall of other individuals regenerate and produce new concentric corpuscles (Jaffe and Plavska, '25). These transplants yield evidence which Jaffe and Plavska interpret as supporting the conclusion of Hammar ('21) that the corpuscles of Hassall (Henle, '46) are nests of hypertrophied reticulum cells of the stroma. Hammar regards these corpuscles as morphologic expressions of an antitoxic activity.

The reticulum cells specified by Hammar in this connection are believed to represent remnants of the entodermal primordium which, during early developmental stages, mingled with the reticulum of mesodermal origin to form the stroma of the definitive organ. At this stage the two varieties of reticulum, entodermal (epithelial) and mesodermal, are indistinguishable in ordinary histologic preparations. In tis-

sue cultures, as described by Popoff ('26), however, the entodermal reticulum of the thymus of the rabbit behaves differently from the mesodermal reticulum. The former grows in the form of an epithelial membrane, the cells of which are non-phagocytic for carmine; the mesodermal reticulum, brought into the thymus with the blood vessels, grows in the form of a syncytium and its cells actively phagocytose colloidal dyes. Popoff is unable to state whether new concentric corpuscles are formed in vitro. Paulizky ('63) and Watney ('82) conclude that the corpuscles arise from connective-tissue cells of mesenchymal origin.

There is a fourth interpretation of the Hassall's corpuscles first urged by Cornil and Ranvier ('73) and by Afanassiew ('77), but consistently ignored by later histologists as without sufficient evidence. Afanassiew came to the conclusion that the thymic corpuscles represent segments of blood vessels, mainly veins, which suffered obliteration through endothelium hypertrophy in the course of involution.

It is the purpose of this reinvestigation to supply further histologic evidence which seems to demonstrate an origin of these corpuscles chiefly from capillaries and precapillary arterioles of involuting thymus bodies in man and the rabbit. The evidence suggests an origin in small part also from reticulum cells, but the precise differentiation of a hypertrophying reticulum cell from an endothelial cell in a similar condition is admittedly difficult and uncertain. Assuming that the reticulum cell in question is actually a mesenchymal representative rather than an entodermal remnant, evidence for which accrues from a study of involuting lymph nodes, a double origin of Hassall's corpuscles from two genetically closely related and only slightly differentiated derivatives of the parent mesenchyme, reticulum cell, and endothelium, implies no serious contradiction. If the explanation of the concentric corpuscles as segments of obliterated blood vessels in the involuting thymus is correct, then it should be easily possible to demonstrate comparable structures in other lymphoid organs in process of regression. Evidence of such

corpuscles we have found in certain involuting subcutaneous and abdominal lymph nodes of a rabbit.

MATERIAL AND METHODS

The material of this investigation includes thymus bodies of man, rabbits, and guinea-pigs, and subcutaneous and abdominal lymph nodes of full-grown rabbits. The staining technique was in every case a combination of hematoxylin and eosin. The human thymi include one from a three-year-old child that died of colitis at the University Hospital. We are indebted to Dr. H. T. Marshall, professor of pathology, for this material. A second thymus is that of a six-year-old child, also from the University Hospital. The thymus of a seven-month fetus and that of a child that died of asphyxia six hours after birth were obtained from St. Elizabeth's Hospital, Richmond, through the kindness of Dr. J. Shelton Horsley, Jr. The material from the University Hospital was fixed in the Zenker-formol solution; that from St. Elizabeth's Hospital in a 10 per cent formol solution.

DESCRIPTION

Typical concentric corpuscles are represented in figures 1 to 5. Figure 1, from the thymus of a three-year-old infant, is of the follicular sort. It consists of a thin laminated capsule and an incomplete core of a generally homogeneous acidophilic material. The core may be locally more or less fibrillated, and include smaller deeper staining spherules and occasional red blood corpuscles. Figure 2 illustrates a variety of corpuscles in which the capsule is very thick, the central portion consisting of lamellated bodies of various sizes. The concentric corpuscles of figure 3, drawn from a section of the same thymus as the preceding figures, each contain centrally a large epithelioid cell. This cell represents a free endothelial cell about which the capsule formed from the wall of the occluded precapillary, and around which as a nucleus may subsequently form laminated spherules as in figure 2. These corpuscles of Hassall are enveloped by relatively small amounts of connective tissue.

Figures 4 and 5 represent corpuscles from the thymus of a seven-month human fetus. They are typical of the solid variety. They consist peripherally of concentric lamellae of greatly flattened epithelioid cells. The center consists of one or several homogeneous laminated spherules. The striking feature in the case of these corpuscles concerns the coarse strands of connective tissue in which they lie. The significant point in this relationship inheres in the fact that these same trabeculae of connective tissue support a blood vessel directly in line with the concentric corpuscles, as shown in figure 5. The close connection between Hassall's corpuscles and blood vessels has been noted by many previous workers (His, '62; Paulizky, '63; Watney, '82; Hammar, '21). His described the corpuscles as growing around the blood vessels, often at the point of branching, and completely enveloping them. Watney interprets the relationship as the inevitable result of the origin of the corpuscles from connective-tissue cells of the trabeculae which supported the blood vessels. According to our interpretation, the attachment of concentric corpuscles to the wall of blood vessels follows from the fact that some arise as areas of luminal occlusion at the point where an arteriole or precapillary branches into smaller vessels.

Figures 6 to 8 represent three successive sections of the same corpuscle from the thymus of a newborn child. Figure 6 shows terminally, at the right, a typical corpuscle of Hassall. At the left is the dilated blind end of a precapillary arteriole. The lumen is filled with blood cells, predominantly lymphocytes. We interpret the corpuscle proper as representing the locus of occlusion of the blood vessel lumen following hypertrophy of the lining endothelial cells. Figure 6, considered by itself, might be taken to support Hammar's explanation of the follicular variety of corpuscles containing lymphocytes, as the result of a secondary invasion by lymphoid tissue (figs. 23, 26, and 30). However, examination of serial sections, as shown in figures 7 and 8, shows clearly that the lymphoid area of the corpuscle of figure 6 leads directly into the blind dilated end of a blood vessel.

If our interpretation of Hassall's corpuscles as areas of segmental obliteration of the lumen of smaller arterial blood vessels following an involution hypertrophy of endothelial cells is correct, then it ought to be possible, as claimed by Hassall ('46) and by Afanassiew ('77), to find similar concentric corpuscles in other lymphoid tissues during involution stages. We have put our hypothesis to the test in the case of certain subcutaneous and abdominal lymph nodes of the rabbit. In these lymph nodes, in which involution is well under way, as indicated by a general disappearance of demarcation between cortex and medulla and the occurrences of areas of fibrosis, hyalinization, and myxoid degeneration, appear certain spheroidal areas of hypertrophied endothelial cells which, together with their enveloping layers of connective tissue, closely resemble the thymic corpuscles of Hassall (figs. 9 to 15). Many of these collections of enlarged endothelial cells enveloped by connective tissue unequivocally represent areas of occlusion in precapillary arterioles, following a primary capillary occlusion or collapse (figs. 9 to 12). Certain similar collections of cells (figs. 14 and 15) apparently represent fusion products of reticulum cells. Their primary origin, however, may perhaps be from one or several hypertrophied endothelial cells of disappearing capillaries, as suggested by figure 45, acting as a focus for the secondary addition and fusion of reticulum cells. Such origin of corpuscles from reticulum of mesenchymal derivation supports our scepticism regarding the genesis of thymic corpuscles from entodermal reticulum, as claimed by Hammar.

Having thus formulated and tested our hypothesis regarding the significance of the concentric corpuscles of the mammalian thymus, we proceeded to a further attempt at verification by a closer study of corpuscles of the unicellular type. It is our opinion that a satisfactory explanation of Hassall's corpuscles has been missed largely through neglect of their earliest stages, and especially through failure to arrange a genetic series between the simpler one-cell types and the large more complex follicular varieties. We will proceed

next to trace this genetic history, and finally again to test our interpretation by tracing in involuting lymph nodes blood vessels in process of obliteration into connection with structures with the general appearance of concentric corpuscles. In other words, in the case of the thymus we will proceed from capillaries and precapillary arterioles to Hassall's corpuscle; in the case of lymph nodes, from concentric corpuscles to arterial blood vessels.

Figure 16 illustrates a typical Hassall's corpuscle of the unicellular variety. This material, as that represented by figures 17 to 30, is from a thymus of a three-year-old child. The large spheroidal cell of figure 16 represents a hypertrophied endothelial cell occluding the lumen of a capillary. To the left appears an endothelial cell at an early stage of hypertrophy. In figure 17 the capillary lumen is apparently occluded by the enlarged endothelial cells. The homogeneous spheroidal mass at the left represents a later stage of regressive change in a cell like that of figure 16.

Figure 18 represents a longitudinal section of a precapillary arteriole bifurcating at its lower end. Both in this region and in that of the opposite end, enlarged endothelial cells have practically occluded the lumen. Near the middle occurs a large spheroidal cell, a Hassall's corpuscle of the unicellular variety. The cytoplasm contains a vacuole within the concavity of the crescentic nucleus. The cytoplasm shows very clearly a characteristic radial striation. It is apparently attached to the larger vessel at the point of an earlier capillary branch. This capillary has apparently collapsed and is presumably in process of resorption. Location of this hypertrophied endothelial cell slightly lateral to its present site would, after medial collapse of the capillary, have placed this Hassall's corpuscle in external connection with the larger blood vessel.

Figure 19 is of a longitudinal section of a precapillary in which the lumen has become obliterated for a considerable distance through endothelium hypertrophy. Transverse section through this region would give structures practically

identical with, though in this case somewhat smaller than, the typical Hassall's corpuscle of the solid type of figure 22. Similarly with regard to figures 20 and 21, transverse sections of precapillary arterioles in which the endothelium is undergoing hypertrophy; a slightly later stage in the process would lead to structures like that of figure 22.

Figures 23 and 25 may be explained on the basis of figure 18. Assuming a complete occlusion of the distal (lower) end of the precapillary of figure 18 and only a partial occlusion in the proximal portion, then the blood stream under pressure in the direction toward the occluded end would transport to and concentrate about the originally unicellular Hassall's corpuscle a collection of blood cells, and so convert the simpler corpuscle into a more complex laminated structure with central amorphous core like that of figure 25. Assuming next a complete blockage of the lumen of the blood vessel of figure 18 in the lower region through the presence of a multicellular corpuscle, then there would occur a region of increased blood pressure proximal to this area of occlusion causing a dilatation of the vessel and an accumulation of cells, and this would result in the formation of a Hassall's corpuscle of the follicular type with lymphocyte content like that of figure 23 (also 7 and 8). Figure 23 is a compound corpuscle of this variety; the smaller solid corpuscle, below, represents the occluded more distal end of the precapillary; the larger follicular corpuscle represents the dilated vessel proximal to the point of occlusion. Figure 24 actually shows the continuity of such a dilated area in a precapillary with the arteriole of normal caliber. Continuation of the process of cell accumulation under pressure in such preocclusion areas leads to corpuscles of the type shown in figures 26 and 30.

The corpuscle of figure 27 also admits of interpretation as a later stage in the development of the corpuscle of figure 18. The only assumption necessary is that the occlusion in the upper portion of the vessel has progressed up to the large spheroidal cell. The practically homogeneous core of the

corpuscle of figure 27 still shows the remnant of the cell nucleus. The larger size of this core, as compared with the cell of figure 18, is the result of peripheral addition in concentric layers of a colloid-like substance, probably a derivative of the involved blood plasma. That such accretions may be derived from such a source is demonstrated in the case of the corpuscle of figure 29 from the same thymus. Here the larger lumen is filled with such a material, probably a blood coagulum, the corpuscle having somewhat the appearance of a follicle of the thyroid gland. Along the right border of the lumen some of the cells are still recognizable as constituents of an endothelium. That the 'colloid' content of this corpuscle is actually a blood-plasma derivative is demonstrated by the fact that certain of the interlobular arteries contain in these sections an identical coagulum. The corpuscle of figure 28 is remarkable for the fact that certain of the cells lining the restricted lumen are of tall columnar shape. These last three illustrations, figures 27, 28, and 29, demonstrate the origin of at least certain of the Hassall's corpuscles of the thymus from occluded segments of blood vessels following local hypertrophy of the endothelium.

The illustrations, figures 31 to 34, were made from sections of a thymus of a six-year-old child. The first shows a longitudinal section of a capillary in a region of endothelial-cell hypertrophy. The erythroplastids are arranged in single file. A transverse section through the area of hypertrophy would yield a structure like that of figure 32, a typical concentric corpuscle of the solid type, closely similar to that of figure 22.

The concentric corpuscle of figure 33 gives information of much importance. Here the relatively small 'colloid' core lies in a lumen lined by a membrane which has the appearance of an unmodified endothelium. The several layers of enveloping epithelioid cells are apparently of reticulum-cell origin. Such corpuscles may be the basis of His' explanation of concentric thymic corpuscles as the result of a growth of connective tissue around a blood vessel. Figure 34 may be

interpreted as the result of a secondary invasion by lymphocytes from the proximal arteriole and a consequent dilatation of a corpuscle like that of figure 33. The strand of colloid material at the left may represent the original core. The capsule of this corpuscle shows a common characteristic of these structures in this thymus and those of the adult rabbit and guinea-pig, namely, the occurrence of basophilic granules and globules in some of the cells (see also fig. 37). These presumably represent later degeneration products.

These degeneration globules are usually abundant in otherwise typical concentric corpuscles in our guinea-pig material. We were especially impressed with this fact, because they at first led to some confusion. We employed guinea-pigs for our attempts to inject the Hassall's corpuscles with Indian ink. The granules, which stain black in hematoxylin, are in general larger than the ink grains, but it was only after an examination of sections from control specimens that it became perfectly obvious that we were in no case dealing with injected ink.

The concentric thymic corpuscles shown in figures 35 to 39 represent conditions in the thymus of an adult albino rabbit. The first illustrates a small corpuscle as an area of occlusion in a capillary, essentially identical with that of figure 18. Figure 36 represents a transverse section of a capillary at a level where the single endothelial cell is greatly enlarged and the constricted lumen contains a single cell. This is a typical small Hassall's corpuscle. The central cell is interpreted as a free endothelial cell; it may possibly be a lymphocyte.

The double corpuscle of figure 37 is chiefly remarkable for the persistence of fairly sharp cell outlines of some of the hypertrophied endothelial cells. From corpuscles like these and that of figure 39, compared with one like that of figure 38, one seemed forced to conclude that the greatly flattened condition of the cells next the central core, especially of the follicular type of corpuscle, is largely the result of the operation of a secondary factor of internal pressure following the

accumulation under pressure of cells and coagulum in a static area proximal to a region of capillary or precapillary occlusion.

Figure 39 illustrates the most remarkable variety of corpuscle found. One is at a loss whether to describe it as a modified blood vessel or a peculiar sort of concentric corpuscle. In fact, it represents actually a stage in the formation of a concentric corpuscle from a larger blood vessel. The lumen contains a large mass of more or less fused erythroplastids, partially surrounded by a homogeneous coagulum (like that of fig. 29). The lining cells are of the columnar variety, with sharp outlines in certain regions. The structure suggests the duct of a gland. However, the presence of blood and coagulum demonstrates its blood-vessel origin; and the presence of similarly modified endothelial cells in more typical concentric corpuscles (figs. 28 and 37) warrants interpretation as a variety of Hassall's corpuscle.

We may now return again to the involuting lymph nodes of the rabbit. Figure 40 illustrates a unicellular form of corpuscle, derivative of a capillary endothelial cell. Figures 41 and 42 represent transverse sections of precapillary arterioles in which the hypertrophied endothelium has almost occluded the central lumen. A later stage leads to the conditions illustrated in figures 43 and 44, typical concentric corpuscles without colloid core. The corpuscle of figure 45 is representative of a large number of variable cell aggregates in these involuting lymph nodes which apparently arise as fusion products of hypertrophic reticulum cells. The frequent presence of one or several central red blood corpuscles suggests that they form about obliterated capillaries, and that some of the cells may be endothelium derivatives. Their chief significance lies in their resemblance to the primordia of concentric corpuscles described by Hammar as having origin from entodermal reticulum. If these corpuscles in thymus and involuting lymph nodes are actually homologous structures, as they appear to be, then their origin in the thymus also may be presumed to be from mesoderm reticulum

(or in part capillary endothelium), as is clearly the case in lymph nodes.

DISCUSSION

Our interpretation of the above-outlined conditions is as follows: Involution of the thymus is initiated by retreat of the terminal capillaries of the lobular medulla. This is correlated with a hypertrophy of certain of the endothelial cells. The point of stenosis of a capillary following endothelial cell enlargement represents the smallest variety of Hassall's corpuscle. Distal to the first point of luminal occlusion, the capillary generally collapses and its cells mingle with the reticular stroma and are presumably resorbed. Proximal to this first level of occlusion, others occur, giving rise to successively larger initial corpuscles. Their peripheral lamellae consist chiefly of reticulum cells. These earlier corpuscles enlarge through continued hypertrophy of their central cells and subsequent additions of reticulum lamellae from the adjacent stroma. The blood stream flows in the direction of the arterial capillaries. When these, and subsequently the arterioles, become locally occluded by the hypertrophic endothelium, the blood pressure rises in the static area and effects a dilatation of the channel proximal to the Hassall's corpuscle. Such dilated areas become converted into the cystoid corpuscles with a considerable content of blood cells. Subsequent changes involve peripheral lamination of the endothelial and reticular cells and a central hyalinization of the blood mass.

This interpretation of the significance of Hassall's corpuscles of the mammalian thymus can be put to the test in involuting lymph nodes. Such nodes should contain similar concentric bodies. As far as we are aware, they have never been reported here. Afanassiew states that Hassall's corpuscles occur in other parts of the body than the thymus, but failed to specify the organ. He quotes Hassall as stating that "similar bodies arise in other parts of the body, but do not lodge in the capillaries, being carried on by the force of the

blood, but in the thymus when the organ starts degenerating the flow is less rapid and these corpuscles lodge in the capillaries here, and finally close them."

We possess certain lymph nodes of the rabbit, from subcutaneous and abdominal regions, in various stages of regression. Here occur modified regions of the precapillary arterioles which fit exactly the above-outlined explanation of the origin of the concentric thymic corpuscles. In these lymph nodes of the rabbit occur segments of precapillary arterioles in which the endothelial cells have become hypertrophied to such an extent as to practically occlude the lumen (figs. 40 to 45). Such areas are closely similar to the smaller and earlier concentric corpuscles. They consist centrally of a core of acidophilic epithelioid cells, including a few red and white blood cells, and peripherally they consist of a layer of variable thickness composed of flattened, concentrically arranged connective-tissue cells.

These nodes do not contain structures comparable to the cystoid corpuscles of the thymus, nor such as include hyaline centers, but these differences we believe pertain to degrees, not character, of development. Moreover, here occur also single large acidophilic reticulum cells, and small collections of similar cells, in every essential respect comparable to the smaller thymus corpuscles described by Hammar as having origin from epithelial reticulum.

The involution specified in the case of the human thymus bodies above described is probably not normal. Hammar ('21) claims that normal (age) involution occurs from the eleventh to the sixteenth year, and that the first concentric corpuscles appear at the 50-mm. stage of development at an age of approximately eighty days. He states further that great individual variation obtains, and that the changes in accidental, inanition, or disease involution of the thymus are similar to age changes; and he describes atretic alterations in the blood vessels during more advanced age involution. According to Afanassiew, Friedleben ('58) mentions the thickening of the wall of the arteries of the thymus with sub-

sequent obliteration, enlargement, and varicose degeneration of the veins during the involution of the organ.

Our procedure, in which we have traced the formation of typical Hassall's corpuscles, both of the solid and follicular types, from their original unicellular or parvicellular conditions, representing hypertrophied endothelial cells in a capillary, and operating then as genetic focus or proximate cause, reveals, we believe, the true significance of these thymic structures. Our conclusion that these concentric corpuscles of the thymus represent mainly and essentially segments of occlusion in capillaries and precapillary arterioles following endothelial cell hypertrophy seems to be confirmed by the discovery of similar corpuscles in involuting subcutaneous and abdominal lymph nodes of the rabbit, where they can be unequivocally traced into relation with blood vessels.

The suspicion will be inevitable, we believe, that we may have confused two genetically distinct structures in the thymus, namely, genuine Hassall's corpuscles and simulacra of these bodies in the form of local areas of endothelial-cell hypertrophy in regressive blood vessels. Hammar and others have previously noted such vascular atresia in the involuting thymus. The alleged demonstrative test of our hypothesis in the case of involuting lymph nodes of the rabbit may be thought to apply only to these atretic areas in the medullary blood vessels of the thymic lobule. The point we wish to emphasize is that there is actually no difference in the thymus between the process of concentric-corpuscle formation and blood-vessel occlusion through endothelium hypertrophy. The genetic relationship between the two has been largely overlooked or ignored. The key to the matter is furnished by the initial stage. The unicellular 'concentric corpuscle' is located intravascularly (figs. 16 to 18). This may secondarily become placed extravascularly through collapse of the containing blood vessel and its separation from the parent vascular stem. When not thus separated, the one or several hypertrophic endothelial cells may completely or partially occlude the arterial capillary. In the event of partial occlu-

sion proximal to a previous complete occlusion or collapse of a capillary, secondary additions of blood cells and plasma, under increased blood pressure in this static area, effect an enlargement of the original corpuscle (fig. 25). In the event of complete occlusion, a follicular form of corpuscle develops proximal to the area of atresia. Such corpuscles consist of only a thin capsule, and their 'lumen' is filled with a blood coagulum containing red blood corpuscles and many lymphocytes (figs. 6, 7, 8, 23, 24, and 26). This type of corpuscle is essentially a dilated blind terminus of a precapillary arteriole (fig. 24), proximal to a region of atresia (fig. 23). The peripheral lamellae consist mainly of additions of reticulum cells.

Afanassiew, in his interpretation of Hassall's corpuscles as obliterated blood vessels, emphasized the predominant involvement of the veins. Possibly some of the smaller varieties may have a venous origin, but certainly the follicular types develop in precapillary arterioles. The blood stasis under pressure essential for their formation does not generally obtain in the veins. Moreover, the unicellular forms always occur in the capillaries. Indeed, these furnish either the focus or proximate cause of formation of the larger multicellular varieties. As the blood vessels become terminally blocked, in the center of the lobular medulla, the circulation is presumably shunted to lower levels. The occurrence of Hassall's corpuscles only in the medulla of the thymic lobule is probably explicable by the fact that the involution process, as indicated also by extravascular features, is initiated in this region.

SUMMARY

1. A large variety of concentric corpuscles of Hassall from the human thymus and that of the rabbit are here described. The chief types include unicellular, multicellular, solid, and follicular forms.

2. The histologic data consistently support the interpretation of these thymic corpuscles as restricted areas of stenosis

or occlusion of the lumen of medullary capillaries and pre-capillary arterioles resulting from endothelial cell hypertrophy concomitant with either disease or age involution. The concentric corpuscles may enlarge either by central (luminal) additions of endothelium, blood cells, or coagulated blood plasma, or by peripheral additions of reticulum or other connective-tissue cells. The follicular forms of Hassall's corpuscles represent essentially blind terminals of pre-capillary arterioles dilated under pressure by the aggregation of blood cells proximal to a region of luminal occlusion.

3. The interpretation of the concentric thymic corpuscles in terms of a segmental obliteration of blood vessels is supported by the occurrence of similar structures, clearly of vascular origin, in certain involuting subcutaneous and abdominal lymph nodes of the rabbit.

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PLATE 1

EXPLANATION OF FIGURES

1 Concentric corpuscle with thin capsule and large amorphous core, containing a number of more compact spherules and one erythroplastid. From the thymus of a three-year-old child who died of colitis. $\times 800$.

2 Corpuscle from the same thymus with relatively thick capsule and central hollow containing three acidophilic spherules. $\times 800$.

3 Two adjacent corpuscles from the same thymus each with a central atrophic cell. $\times 800$.

4 Large corpuscle at the end of a coarse strand of connective tissue. From the thymus of a fetus of the seventh month. $\times 800$.

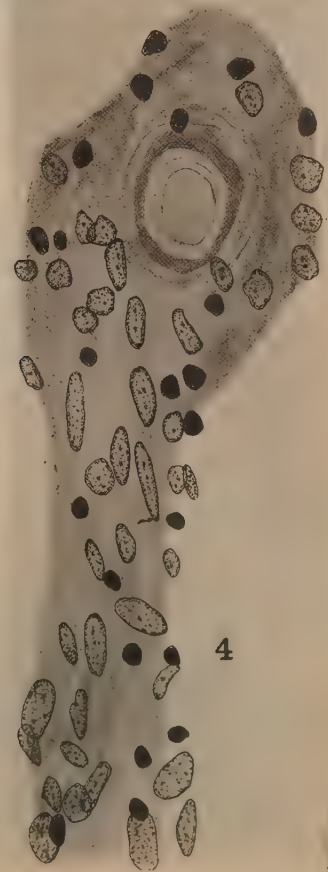
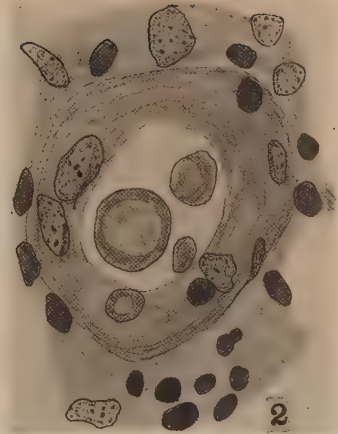
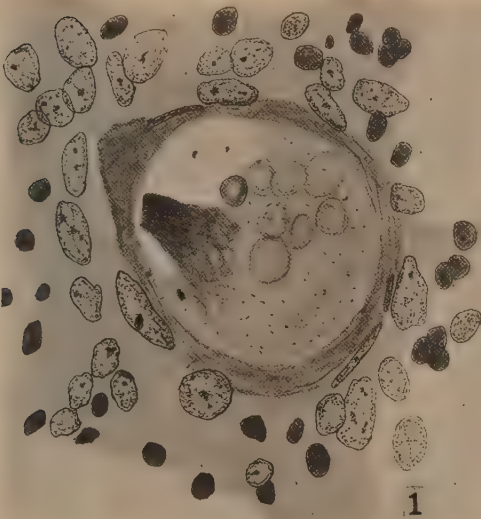


PLATE 2

EXPLANATION OF FIGURES

5 Two corpuscles in the same coarse strand of connective tissue, containing (below) the end of a precapillary artery. Fetus of seventh month. $\times 400$.

6 to 8 The same corpuscle in three successive sections, showing its relation to the dilated end of an occluded arteriole. Thymus of a newborn, dead of asphyxia six hours after birth. $\times 400$.

9 Body resembling a less advanced Hassall's corpuscle, from a subcutaneous lymph node of an adult brown rabbit. This body represents an occlusion of a precapillary artery following hypertrophy of its endothelium. At the lower end appears the lumen of the artery with several erythroplastids. $\times 800$.

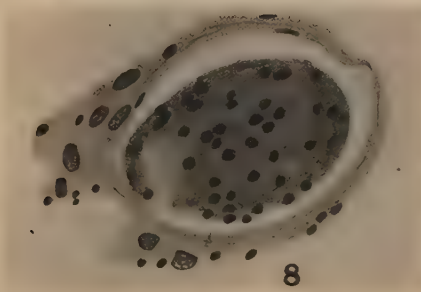
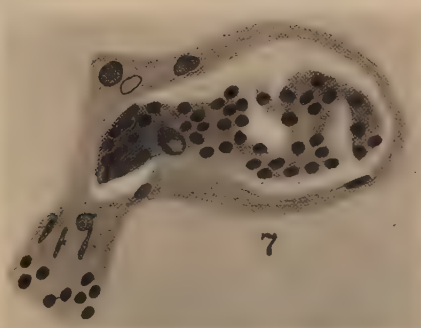
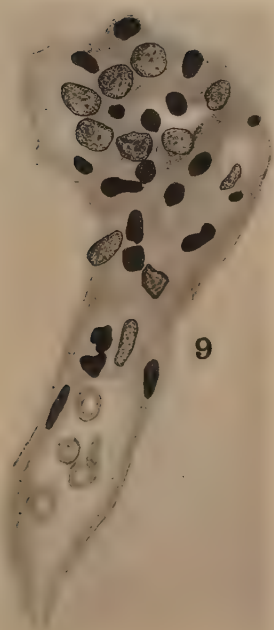
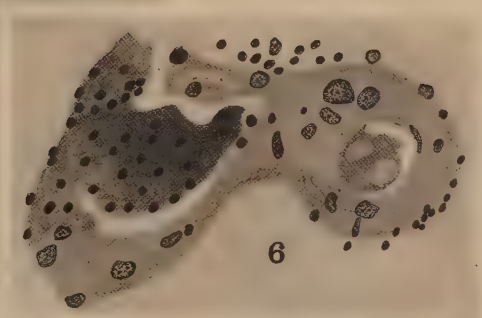
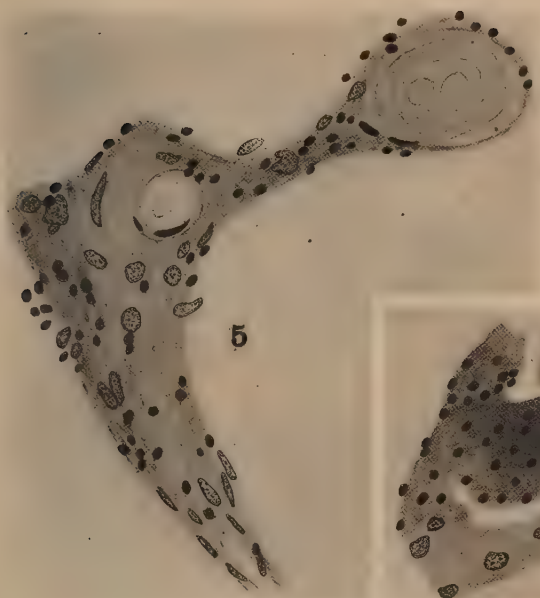


PLATE 3

EXPLANATION OF FIGURES

10 to 12 Similar concentric corpuscles from a lymph node of the mesocolon of the same rabbit. $\times 800$.

13 to 15 Successively larger corpuscles from the lymph node of the mesocolon of the same rabbit. These have originated from fusions of reticulum cells, probably around collapsed atrophic capillaries as centers. $\times 800$.

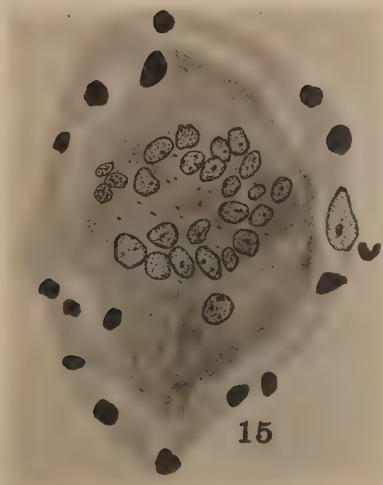
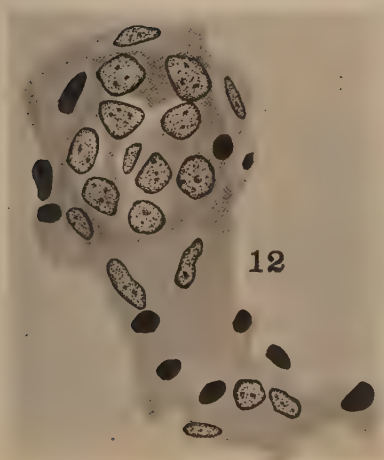
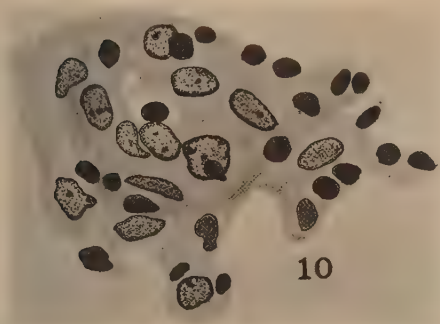


PLATE 4

EXPLANATION OF FIGURES

16 Typical unicellular Hassall's corpuscle. It lies within the lumen of a capillary. At the left appears an endothelial cell in process of hypertrophy. Thymus of three-year-old child. $\times 600$.

17 Capillary from same thymus, showing occlusion by a Hassall's corpuscle consisting of a homogeneous acidophilic globule. $\times 600$.

18 Longitudinal section of a precapillary artery, showing two regions of occlusion by hypertrophied endothelial cells and a central single-cell Hassall's corpuscle.

19 Longitudinal section of occluded portion of a capillary. A transverse section of this area would give the typical appearance of a small Hassall's corpuscle.

20 and 21 Transverse sections of a smaller and larger precapillary artery from the same thymus, showing considerable hypertrophy of the endothelium, with the general appearance of concentric corpuscles.

22 Typical small solid concentric corpuscle of Hassall. Compare with figure 21, in which a portion of the original lumen of the blood vessel persists.

23 Concentric corpuscle with central lumen containing, within the homogeneous acidophilic mass, granulocytes, lymphocytes, and erythroplastids. Below is shown the completely occluded blood vessel. The larger of the pair of associated corpuscles represents the dilated end of the arteriole proximal to the point of first occlusion (represented by the smaller solid corpuscle).

24 Similar follicular concentric corpuscle, continuous at the right with the open arteriole, representing the dilated end of the blood vessel proximal to the point of occlusion.

25 Concentric corpuscle with small central laminated acidophilic (hyaline) core, enveloped by a fused mass of leucocytes and red blood corpuscles. Its peripheral capsule is unusually thin. This type of corpuscle represents a condition of only partial occlusion or secondary reopening under blood pressure of the arterial lumen, permitting thus a secondary collection of blood cells about the original nucleus of hypertrophic endothelial cells.

26 Follicular or cystic type of Hassall corpuscle with thin capsule and luminal coagulum containing many blood cells. The capsule contains a capillary (below). This corpuscle represents a greatly dilated blind end of a precapillary artery, proximal to the point of complete occlusion. Thymus of three-year-old child. $\times 600$.

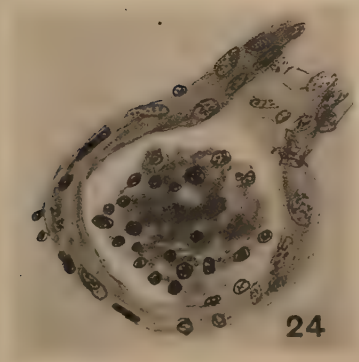
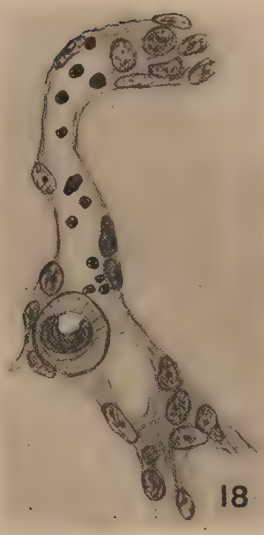
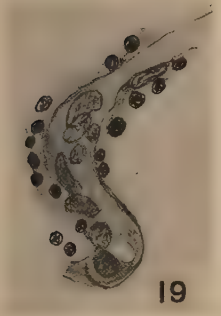


PLATE 5

EXPLANATION OF FIGURES

27 Small Hassall's corpuscle within the occluded portion of a precapillary artery. A transverse section through the central portion would resemble figure 25; through the lower area of occlusion, that of figure 22. Thymus of three-year-old child. $\times 600$.

28 Concentric corpuscle with central lumen from same thymus. Large endothelial cells line the lumen. Compare with figures 34 and 37.

29 Corpuscle whose large central lumen contains a homogeneous mass of an amorphous acidophilic material resembling colloid. Certain large arterioles in the interlobular connective tissue of this thymus contain a similar substance in addition to blood cells. It probably represents a blood coagulum. Compare with figures 34 and 37. $\times 600$.

30 Large follicular corpuscle with thin capsule and amorphous acidophilic content. Below, at the right, the central mass contains various types of blood cells. This corpuscle may be interpreted in terms of a greater dilatation, in consequence of greater blood pressure proximal to the area of complete occlusion, as compared with corpuscle, figure 29. Thymus of three-year-old child. $\times 600$.

31 Longitudinal section of a capillary, showing a constricted lumen, with erythroplastids arranged in single file, and an area of endothelial cell hypertrophy at the upper end. Thymus of six-year-old child. $\times 600$.

32 Small concentric corpuscle of Hassall from the same thymus. Its minute central lumen contains a single erythroplastid. This corpuscle represents essentially a transverse section through the upper end of the corpuscle in figure 31. $\times 600$.

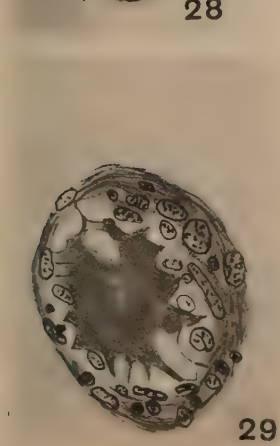
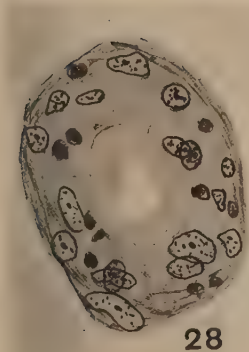
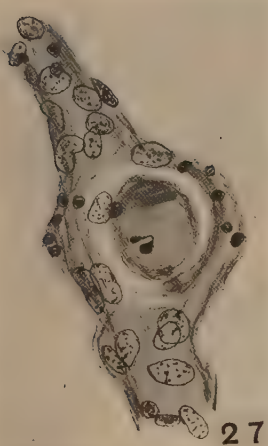


PLATE 6

EXPLANATION OF FIGURES

33 Larger concentric corpuscle with colloid mass in lumen. The peripheral cells represent hypertrophied endothelial cells.

34 Similar concentric corpuscle, secondarily dilated through accumulation of blood cells under pressure. The capsule is accordingly thinner. Above, at the left, appears a collection of deeply staining (basophilic) globules, characteristic of many of the corpuscles of this thymus of a six-year-old child. $\times 600$.

35 Small Hassall's corpuscle at dilated end of an occluded capillary. From the thymus of an adult rabbit. $\times 600$.

36 Transverse section of a capillary from the same thymus. The single endothelial cell is greatly hypertrophied. The lumen contains one cell at this level, probably a free endothelial cell. The whole appearance is that of a small Hassall's corpuscle. Compare with figures 16 and 17.

37 Two adjacent corpuscles from the same rabbit's thymus, each with a central lumen. The lumen of the smaller corpuscle contains an atrophic cell; that of the larger contains a coagulum with many blood cells. Certain of the hypertrophied endothelial cells have assumed a columnar shape. $\times 600$.

38 Large follicular corpuscle from the same thymus. The lumen contains a coagulated mass of red and white blood cells. Below, at the right, appear large spheroidal (endothelial) cells. Compare with figures 6 and 8.

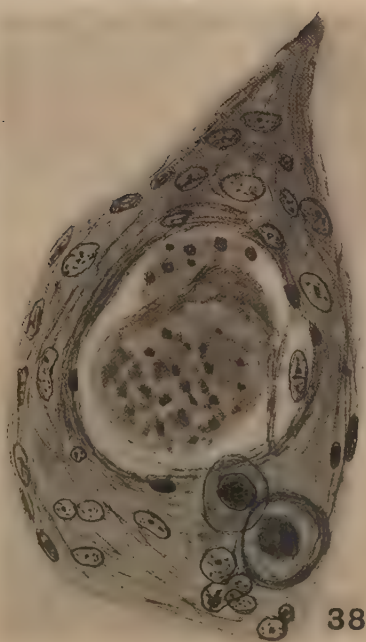
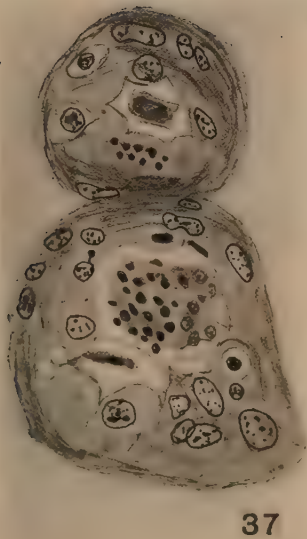
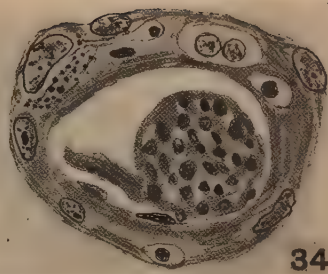
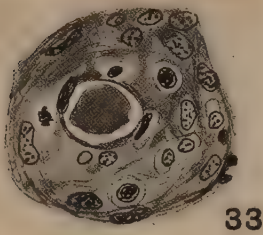


PLATE 7

EXPLANATION OF FIGURES

39 A unique type of follicular Hassall's corpuscle from the same rabbit's thymus. The hypertrophying endothelial cells have assumed columnar shapes. The lumen contains a homogeneous coagulum (below, at the left) and a mass of fused red and white cells, at the right. Compare with figures 28 and 30.

40 Transverse section of a partially occluded capillary, from a subcutaneous lymph node of a rabbit. The enlarged occluding endothelial cell resembles a Hassall's corpuscle of the unicellular type. Compare with figures 16 and 17. $\times 600$.

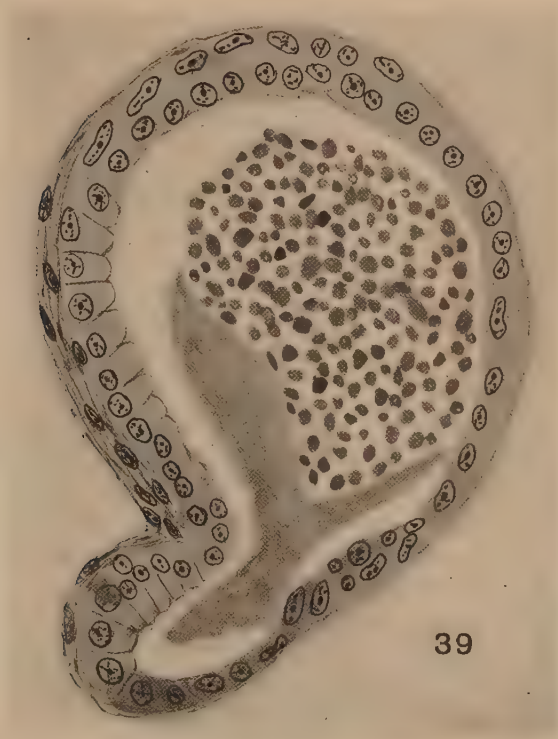
41 Transverse section of precapillary arteriole from a subcutaneous lymph node of a rabbit. The minute lumen, constricted by the hypertrophy of the endothelium, contains a single erythroplastid. Compare with figure 20. $\times 600$.

42 Similar corpuscle of larger size. Compare with figures 2, 32, and 33. Such lymph-node corpuscles, of undoubted blood-vessel origin, are practically indistinguishable from thymic corpuscles like the one in figure 32.

43 Longitudinal section of occluded portion of a precapillary artery from a rabbit's subcutaneous lymph node. Transverse sections would yield appearances like that of figure 42. Compare with figures 16, 17, 18, 19, 31, and 35.

44 Concentric corpuscle from a lymph node of the mesocolon of an adult brown rabbit. This represents an obliterated precapillary arteriole, the result of endothelium hypertrophy. Compare with figure 22.

45 Irregular corpuscle from the same lymph node, formed apparently by the fusion of reticulum cells around the lumen of a capillary. The lumen contains two erythroplastids. Possibly several of the cells represent hypertrophied endothelial cells. $\times 600$.



GOLGI APPARATUS AND VACUOME

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TWO FIGURES

A little over a year ago, I published in this journal a review of certain problems connected with the Golgi apparatus in which the views of Parat and his associates were dealt with in a rather summary manner (Bowen, '26 a). In concluding a reference to the matter I said (pp. 162-163): "A destructive criticism of other features of this theory is relatively easy, but, pending the publication of more detailed results, it seems useless to pursue the matter. The results of Parat and Painlevé are certainly not now acceptable to the majority of workers on the Golgi apparatus." I must frankly say at the outset that my opinion of the validity of Parat's theory has not meanwhile undergone any change, nevertheless a not inconsiderable group of active workers is now looking with some favor upon the theoretical implications of what I will call 'the vacuome theory.' This group includes a number of French authors, both zoological and botanical, together with several English and American workers to whom various aspects of the theory have proved attractive. It is inevitable that a fundamental clash of opinion must develop, which will necessarily involve the whole problem of cytoplasmic structure in both plants and animals. It seems to me, for this reason, desirable to review the whole matter, suggesting various possibilities for the solution of the problem as a whole. Thus, perhaps, much useless contention may be avoided and at the same time the disinterested biologist may readily be given a general understanding of the nature of present-day difficulties in the cytoplasm.

THE STRUCTURE OF ANIMAL CYTOPLASM

It is, I take it, unnecessary to do more than briefly restate the essence of the view now generally held by cytologists as to the morphology of animal cytoplasm. According to this view, there are two formed elements universally present in animal cells—the mitochondria or chondriosomes, forming in their cellular totality the chondriome, and the Golgi apparatus or Golgi material. These substances form perfectly definite aggregates which are quite distinct from the general protoplasmic background in which they are suspended. This protoplasmic groundwork is presumably in the nature of a complex colloidal mixture which with our present microscopical equipment and methods appears absolutely homogeneous to the eye. Chondriosomes and Golgi material, on the other hand, appear in characteristic shapes and arrangements. They are supposed to be absolutely distinct from each other and (by some writers) to possess the power of independent growth and multiplication. Whether they ever arise *de novo* is not definitely known and in any event immaterial for the present discussion. It has been generally supposed that the basis of both these cytoplasmic components is of a lipoidal or fat-like nature, but the evidence, particularly in the case of the Golgi substance, is equivocal, and it is perhaps not particularly important for present purposes what the actual chemistry of these materials may be. The chondriosomes take the form of granules, vesicles, rods, threads, and even rings, the shape being more or less characteristic for particular animals and tissues. The Golgi apparatus appears in the form of discrete bodies of various types and more typically in a so-called reticular arrangement. This latter condition early led to a conception of the Golgi apparatus as consisting of a mass of anastomosing threads, forming a sort of network—a conception which was readily extended to include the idea of a system of canals. Although the occurrence of scattered Golgi bodies would seem to have been sufficient evidence to refute such canalicular theories, they nevertheless attained considerable vogue and are still occasionally

appealed to. Recent workers, as a rule, have been inclined to look in other directions for an explanation of Golgi structure. My own view (Bowen, '26 a, g), in support of which some evidence from other sources is accumulating, is that the Golgi apparatus is primarily a specific substance, the arrangement of which in a cell is subject to endless variation. The appearance of a thread-like or canal-like structure is, in my view, too often an expression of our inadequate technique, and the more nearly perfect the result, the less like a true thread-work does the apparatus become. This is a matter of much importance, in view of certain comparisons which have been drawn, on the supposition that the Golgi apparatus is essentially a system of canals. I believe it fair to say that at present such a view would be acceptable to very few active students of the animal Golgi apparatus.

In addition to the chondriome and Golgi apparatus, animal cells frequently contain other and more or less temporary formed elements, as, for example, secretory granules or droplets. I have suggested (Bowen, '26 g) that the primary basis of a secretory granule may actually be a vacuole within which the granule proper is elaborated, yielding the appearances familiar to every student of gland cells. Such vacuoles are, however, generally looked upon as bound up with the secretory process and have not, as a rule, been considered as part and parcel of the permanent cell system. It is exactly on this point that Parat and others have urged a different view, as will later be shown.

THE STRUCTURE OF PLANT CYTOPLASM

When we turn our attention to the structural elements of plant cytoplasm, we find ourselves at once in the midst of what certainly seems like confusion. A prolonged study of the problem leads me to the conclusion that this confusion is more apparent than real, and if one for the moment avoids labeling things with the names invented by animal cytologists, it appears that a reasonable consensus of opinion might readily be reached. As a basis for discussion I have selected

the results of P. A. Dangeard and of Guilliermond, who (with their pupils) are the only ones in recent years to attempt a generalized analysis of plant cytoplasm. Apparently, they have reached results which are often in conflict, but if one ignores the nomenclatures and concentrates on the morphological facts, it is clear that all have seen the same thing. They differ chiefly in that Dangeard has invented terms which are purely descriptive, while Guilliermond has attempted to translate these terms into animal-cell equivalents.

Considering first the results of Dangeard ('24), it appears that plant cytoplasm contains three classes of formed elements. The first is the plastidome, comprising bodies or plasts of various shapes which play rôles in the construction of leucoplasts, chloroplasts, etc. The second is the cytome (formerly called the spherome), comprising a collection of spherical or slightly elongate bodies called cytosomes (formerly called spherosomes and later microsomes). Both plastidome and cytome are demonstrated by Fe-hematoxylin after proper technique. Finally, there is the vacuome, consisting of the 'ordinary,' well-known plant vacuoles and in very young cells of the 'elementary' vacuoles from which the vacuoles of older cells eventually arise. It has been particularly the work of Dangeard (and later of his son) to elucidate the vacuolar system. It was found that vital stains, especially neutral red and cresyl blue, would stain the vacuoles of plant cells with the greatest selectivity and clearness. The Dangeards have thus been able to demonstrate the occurrence of vacuoles in plant cells of all kinds, including the earliest meristem, where it had long been a botanical tradition that none occur. This work shows that the vacuolar system may assume the most varied shapes, some of which are so bizarre as to arouse in certain quarters skepticism as to the results as a whole. That this kind of criticism is without foundation seems, however, amply proved by the researches of Guilliermond ('13 a, b, c) and more particularly of Pensa, begun, indeed, before those of Dangeard. Pensa's ('13) first paper is of peculiar interest because it deals with the vacuome studied

in living cells where the contents of the vacuoles were stained red by the natural presence of anthocyanin. A more extraordinary setting (suggested by Guilliermond ('13 a)) for the intra-vitam study of a cytoplasmic element has never been devised, and Pensa was able in an individual cell to follow changes in the morphology of the vacuome exactly comparable to the remarkable pictures subsequently obtained by others with vital dyes and silver or osmic impregnation. The vacuoles appear never to arise *de novo* (?), but are permanent elements of the cell handed on in cell division and in reproduction, and are presumed to have a status comparable to that of the nucleus, plastidome, and cytome. In addition to these three permanent and universal components of the plant cell system, Dangeard also recognizes the presence (as in animal cells) of materials of no general significance, as, for example, the fat-like droplets which frequently occur in plant cells.

Guilliermond ('24), for his part, finds three components universally present in plant cells, viz., the chondriome, the vacuome and the lipoidal granulations. The last-named is not given a status equal to that of the first two constituents, and is probably equivalent to the fatty droplets just noted in Dangeard's system. The lipoidal granulations are indeed looked upon merely as products of cellular activity. Guilliermond ('24) believes that these granulations are what Dangeard calls the cytosomes (spherosomes), but the staining reactions described by Dangeard make such a comparison quite impossible. In my own studies on plant protoplasm (now in preparation for publication) I have not found lipoidal granulations as a universal part of the plant cell, and I am inclined to the opinion that Dangeard's own statement of the case is correct. His cytosomes are not the lipoidal granulations, and all observers agree that the latter are not in any event important components of the cell system. It would seem perfectly fair to compare these fat-like deposits with the fat often occurring in animal cells, considering them as of quite secondary importance for an understanding of the permanent and universal parts of the cell system.

As to the general morphology of the vacuome, Guilliermond and the Dangeards are now in essential agreement. As regards, however, the interpretation of the vacuolar system, Guilliermond has developed the view that the vacuome is the equivalent in the plant¹ of the animal Golgi apparatus. This idea was first suggested by Bensley ('10), on the supposition that the Golgi apparatus was a canalicular system. Hinted at by Guilliermond in 1920, in 1922 this view was proposed in concrete form by Guilliermond and Mangelot ('22 a, b) (see also Sanchez ('22 a, b, c), and subsequently Luelmo ('23)). The matter has been followed up, however, only by Guilliermond, who has reiterated his belief in several recent papers. Guilliermond finds the real basis for his view in the fact that silver nitrate will sometimes blacken the vacuome as it does the animal Golgi apparatus, and that at some stages in the development of the vacuome a network is formed which is certainly very like the preparations of the apparatus which one often receives in many vertebrate cells. Subsequently, Guilliermond ('26 d) applied the osmic-impregnation methods to plant tissue, and obtained positive results which greatly strengthened him in his conclusions. I had previously² (Bowen, '26 b) published a report of similar work on plant cells, in which the same morphological results had been obtained. The similarity existing in some cells between the most recent accounts of the morphology of the Golgi apparatus in animal cells and the actual pictures obtained in plant cells (particularly the periblem of *Vicia*) is so extraordinary that, in spite of my earlier views, I was inclined for a while to accept the vacuome, or some part of it at least, as the Golgi apparatus. Further study of the problem indicated that such a comparison was impossible. In the first place, only occasionally does

¹ A paper by Laburu ('16) on the Golgi apparatus in *Solanum* is one of the earliest studies of this problem in plant tissue. Laburu demonstrated characteristic networks by means of Cajal's silver method for Golgi material, and called them the Golgi apparatus. These networks, it is now clear, are the usual vacuolar complexes, but Luelmo did not so refer to them.

² See, however, an indefinite reference to the use of "la méthode à l'osmium réduit" by Guilliermond ('26 a).

the vacuome present a really striking morphological similarity to the Golgi apparatus and in some species such similarity seems always to be lacking. But further trials soon showed that in different plants the results differed among themselves, the blackening occurring in such a way as to indicate that it was the result of the particular contents of the vacuole and had no necessarily general significance. Finally, my experiments showed that osmic acid, while rather specific when properly applied to the Golgi apparatus of animals, in plant cells is capable of blackening almost anything. The similarities of morphology and staining reaction sometimes manifested by vacuome and true Golgi apparatus, therefore, seem to be of the most superficial possible order, and since the supposed canalicular nature of the Golgi apparatus has fallen to the ground, the basis for comparison with a vacuolar system becomes extremely tenuous. It seems to me, therefore, highly precarious at the present time to compare the vacuolar contents with the Golgi apparatus, in the sense that Guilliermond seems to have done. The vacuolar contents are watery, and there is no good reason to suppose that this fluid has any chemical affinities common to the animal Golgi apparatus. This conclusion distinctly does not rule out the possibility of some relation between the walls of the vacuoles and the Golgi material—a possibility which has not, however, been taken into consideration as yet by Guilliermond. It should, in conclusion, be carefully noted that the development of this view by Guilliermond differs slightly from the vacuome theory of Parat, as will appear more clearly in a later section. Guilliermond in essence holds that the vacuole contents of a plant cell are equal to the contents of a comparable canalicular system (Golgi apparatus) in an animal cell. In both cases it is the contents of a specific series of cavities which are blackened by impregnation methods. An essentially similar view of the Golgi apparatus has recently been developed by Corti ('24, '25), who refers to the 'lacunar apparatus of Golgi' or lacuome (lacunoma) as something essentially like the old canalicular channels of Holmgren's

trophospongium theory. The Golgi apparatus, according to Corti, is a congeries of 'lacunae.' This attempt to return to a viewpoint generally abandoned by animal cytologists has not yet received any support except from proponents of some aspect of the vacuome theory.

Finally, Guilliermond finds in all plant cells the chondriome, an assemblage of bodies all of which have the same staining reactions and are presumed to be basically identical. But this chondriome is divided into two parts, 'active' and 'inactive.' The inactive chondriome is typically granular, and has no function which is expressed in visible changes of morphology. Dangeard considers these granules to be the same as his cytosomes (sphaerosomes), and in my opinion this is a correct identification. The active chondriome, on the other hand, consists, in meristem cells, of elongate, often thread-like bodies, from which the various types of plastids are gradually evolved. These bodies are equivalent to the plastidome of Dangeard. It is at present generally held that neither of these components has any relation to either Golgi apparatus or vacuome—a view in which I now concur.

It will be seen, therefore, that while superficially the views of Guilliermond and the Dangeards differ in some apparently vital respects, nevertheless to the unbiased observer the differences are largely a matter of words. Each finds three essential components the morphology of which at least is distinctive. While Dangeard makes no attempt to compare his findings with the animal cell, Guilliermond reduces his to a direct comparison, in which it appears that the formed elements in plant and animal cells are identical. In animal cells there is the chondriome and Golgi apparatus, in plant cells the chondriome (divided into active and inactive constituents) and vacuome (= Golgi apparatus). This reduction of the plant components to two essential ones is of considerable importance in the light of the postulates of Parat's vacuome theory, as will presently be clear.

In the foregoing statement of the problem, I have been particularly guided by my own researches on plant cells which

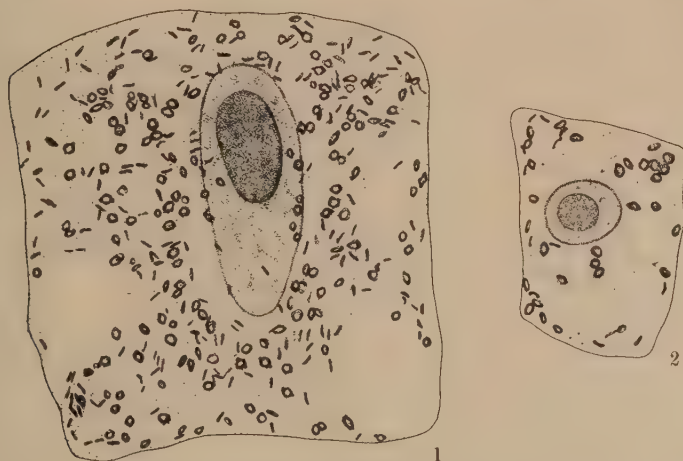
have steadily been pushed for the last year and a half. A preliminary statement of my results is now in press (Bowen, '27). Entering the field of plant cytology without any particular bias as to the possibilities in prospect, I attempted to apply to plant cells those methods which have long been familiar to animal cytologists. In spite of some earlier mistakes in interpretation, the matter has gradually become clearer and I am glad to find myself in the end in substantial agreement with Guilliermond and Dangeard.

I have found, in the first place, the same three general components described by Guilliermond and Dangeard, my general conclusions as to morphology coinciding almost exactly with the findings of Guilliermond. There is, in the first place, the plastidome, a group of bodies always distinct and recognizable even in the promeristem. They are especially characterized by their frequently elongate, sometimes thread-like shape. From these bodies develop the leucoplasts, for example, in root tips. In the second place, there is a collection of small bodies, vesicular in structure, which in meristem are spherical or slightly elongate and easily separable from the plastidome by shape, structure, and, to a certain extent, staining capacity. I have called these bodies tentatively the pseudochondriome, in order to indicate their close resemblance to the animal chondriome, without committing myself finally to such an identification. It is clear that these are Guilliermond's inactive chondriome and Dangeard's cytosomes (sphaerosomes). In my first report on plant cells (Bowen, '26 b), these bodies were erroneously grouped in part with the plastidome, due to the fact that they rarely impregnate with osmic acid, the hyacinth root tip being a distinct exception. This confusion led me to suggest that in the moss sperm (Bowen, '26 c) proof was at hand of the chondriosomal nature of the plastidome. It is now clear that the evidence really points to the pseudochondriome as the equivalent of the animal chondriome, leaving the status of the plastidome still unsettled. It is quite possible that the plastids may turn out to be a specialized part of the plant

chondriome, as Guilliermond asserts, but at present there is evidence perhaps as good to indicate that the plastidome is a cytoplasmic component of plant cells on a footing as independent as that enjoyed by the vacuome and pseudochondriosomes (cytosomes, sphaerosomes, or inactive chondriome). It is clear, therefore, that Guilliermond, Dangeard, and myself are in essential agreement as to the facts about the plastidome and cytome, though entertaining widely divergent views as to their interpretation. I should add that in my review of the Golgi apparatus (Bowen, '26 a) I offered the admittedly rash suggestion that possibly in one of these two components was to be found the equivalent of the animal Golgi apparatus. This suggestion was made in ignorance of the behavior of osmic acid in plant cells and on the supposition that plant cells had been so much studied that all the fundamental structural elements must of necessity have been at least observed and described. For a time (Bowen, '26 b, c) I thought one alternative suggested by me as to the Golgi apparatus in plants was correct, but it became gradually clear (Bowen, '26 d) that this could not be the case, and there can now be no reasonable doubt that neither plastidome nor pseudochondriome is in any way related to the Golgi material.

As a matter of fact it has become evident that there exists in plant cells of many kinds (probably universally) certain bodies which seem not to have been previously described. These bodies are circular platelets, the peripheral rim of which blackens intensely in osmic acid applied according to the technique particularly of Kolatchev. These bodies I have called temporarily the osmiophilic platelets (figs. 1 and 2), because, of all the structures in the plant cell, they are most readily and universally blackened by the osmic Golgi apparatus methods. They are, indeed, never stained by any of the usual cytological methods (except in germinal tissue such as the moss antheridium), and this is clearly the reason for their not having been long ago described. In my first papers (Bowen, '26 b, c) I called these bodies sphaerosomes, trying to classify them with the granular pseudochondrio-

somes which I had demonstrated with acid fuchsin in root tips, but it became gradually evident that such an identification was impossible, and it is now clear that the osmiophilic platelets constitute a cytoplasmic component of plant cells probably on an equal footing with the three other components previously identified by Dangeard and Guilliermond. Study of the behavior of the platelets in moss-sperm formation (Bowen, '26 c, '27) has led me to the belief that these bodies



Figs. 1 and 2 Meristem cells from root tips, showing the osmiophilic platelets as demonstrated by the method of Kolatchev. Figure 1, large cell from the central core of the barley root tip; figure 2, from root tip of the kidney bean.

are connected with the formation of the so-called apical body in the moss sperm, and are accordingly the homologue of the animal Golgi apparatus. This conclusion is further validated by their similarity in staining behavior to the Golgi apparatus of animal cells, by their morphological similarity to the Golgi bodies in insects, and by the probability that, like the animal Golgi apparatus, they are rarely if ever visible *intra vitam*.

With respect to the vacuome, my morphological findings agree substantially with those of the Dangeards and Guilliermond.

The morphological constituents of plant protoplasm may therefore be grouped, with respect to equivalence to each other and to possibly similar elements in animal cells, as follows:

Dangeard Plant	Guilliermond		Bowen	
	Plant	Animal	Plant	Animal
Plastidome	Chondriome { Active (plastidome) Inactive	Chondriome	Plastidome	?
Cytome (sphérome)			Pseudochondriome	Chondriome(?)
Vacuome	Vacuome	Golgi apparatus	Vacuome	?
—	—	—	Osmiophilic platelets	Golgi apparatus (?)
Fatty deposits	Lipoidal granulations	Lipoidal granulations	Fatty granules	Fatty granules

This table will serve to recapitulate the complicated differences of opinion reviewed in the preceding pages and will give at a glance some idea of the most recent views as to the structure of plant cytoplasm. It may be thought by some to be open to the criticism of settling too easily the long dispute about the occurrence, morphology, origin, etc., of the plastidome (proplastids) in early meristem. To such a criticism I can only reply that the evidence seems overwhelming as to the occurrence of the plastidome and cytome as distinct, independent, and universal constituents of plant cytoplasm. The long controversy on this point has, in my opinion, grown largely out of inadequate technique and failure to study the problem along sufficiently broad lines.

The foregoing discussion of the structure of animal and plant cytoplasm may have seemed quite irrelevant to the problem in hand; but, as a matter of fact, a knowledge of the pros and cons of cytoplasmic morphology is absolutely essential to any critical consideration of the vacuome theory

and particularly the difficulties which it seems to encounter. To this theory we may now finally address ourselves.

THE VACUOME THEORY

The first definite step toward the vacuome theory was suggested in 1924 by Accoyer. Working with vital dyes on the choroid epithelium of the white rat, he succeeded in applying both neutral red and Janus green to the same cell. The result was the demonstration of the chondriome in green and the secretory granules (or vacuoles around the granules, as noted previously) in red. Accoyer concluded that these results brought a new proof to the thesis that chondriome and vacuome were independent cellular structures—a view in accord with that of Guilliermond concerning plant cells. It will be recalled that in Guilliermond's view there are only two essential components in plant cytoplasm, chondriome and vacuome. The matter was presently taken up by Parat³ and several coworkers ('24 a, etc.), particularly Painlevé, and in a long series of papers the vacuome theory has gradually been evolved.

This theory postulates as a foundation stone that there are present in all cells, both plant and animal, two and only two kinds of fundamental formed elements in the cytoplasm. They are distributed in a protoplasmic background which is homogeneous and, to the ultramicroscope, optically empty. The elements in question are, first, the chondriome, stainable *intra vitam* (?) by Janus green, and, second, the vacuome, similarly stainable with neutral red. Indeed, the vacuome is itself defined as the ensemble of vacuoles stainable by neutral red. In other words, it would appear that Parat looks upon Janus green and neutral red as specific stains in a rather narrow sense. Those structures which stain with Janus green

³ I have continually referred to the 'vacuome theory of Parat.' It will certainly be clear to the reader that others, especially Painlevé, have collaborated with Parat in the elaboration of this theory, but it is evident that Parat has been the leading spirit in its development. It seems, therefore, fair to refer to the theory under Parat's name alone merely as a matter of convenience.

form, by definition, the chondriome (Janus green may also stain the vacuoles to some extent), and those which stain in neutral red form, by similar definition, the vacuome. The formed elements of all cells must fall into one or the other of these two classes, the former being a lipoidal phase and the latter a watery one—whence the staining reactions. This last statement is, of course, meant to refer only to the permanent and generalized formed elements in cytoplasm, and would not include fat-droplets, crystals, and other ‘ergastic’ materials of temporary or specialized significance. Since the use of the term chondriome as here defined is quite similar to that of Guilliermond, we may dismiss this component and center attention on the vacuome.

The first essential characteristic of the vacuome is, then, its specificity for neutral red. Parat postulates that all living cells possess a vacuome, which may be invariably demonstrated in fresh material by neutral red. The second essential characteristic of the vacuome is, therefore, its constancy. The third essential characteristic is the nature of the vacuolar contents, which are never lipoidal, but always watery with a reaction described as ‘franchement acide,’ and probably made up in most cases of a solution of crystalloids. Characteristics of minor importance are those of position in the cell and of dimensions, each of which may present various degrees of constancy or variability, but which in any particular case keep within well-marked limits and are definite topographical features of the cell.

The second phase of the vacuome theory has to do with the relation of the vacuome to the Golgi apparatus particularly in animal cells. It will be recalled that Guilliermond⁴ has asserted the homology of the plant vacuome with the animal Golgi apparatus largely on the supposition that the latter is

⁴I find it very difficult to make a satisfactory statement of the views offered by Guilliermond and by Parat, inasmuch as they appear at times to agree and at other times to disagree. Guilliermond and Corti seem to hold essentially similar views, looking upon the Golgi apparatus as a vacuolar or lacunar system of almost any shape (except that Corti believes that where networks exist, they are of a very simple order). The demonstration of this lacunar system is due

a canalicular system whose contents stain as do the contents of the plant vacuoles. In its simplest form this view is partially rejected by implication by Parat who goes a step farther and concludes that the Golgi material of animal cells as usually described does not exist. The appearance of a Golgi apparatus results from the fact that within, around, or between the vacuoles of a cell, osmic acid or silver nitrate is reduced to form an entirely artificial structure which, going now this way now that, produces that illusion of a rambling, indefinite network so familiar to students of the animal Golgi apparatus. The blackening of the plant vacuome is thus in many cases of an order quite different from the blackened, artificial deposits which we call the Golgi apparatus in animal cells. Parat is strengthened in this conclusion by the result of tests which he and Bergeot ('25) conducted on the chemical nature of the animal Golgi apparatus. They trace the origin of the idea that the Golgi material is essentially lipoidal to Cajal ('14) (this view is, of course, much older, see, for example, v. Bergen, '04; Sjoevall, '06, and Mulon, '12), and state that none of the usual microchemical tests for fats will give a positive reaction (e.g., Sudan III, Scharlach, indophenol blue, Ciaccio, and Dietrich). With Dietrich the chondriome is stained and the cellular background is darkened, the vacuome appearing on this background as light areas. Hence the conclusion that the classical Golgi apparatus is not formed at all of fat-like materials. The only thing

to the direct blackening of its contents, for example, in osmic acid. Parat looks upon the vacuome as composed of spherical units, and wherever a net has been described, we are actually dealing with an artificial running together of the vacuoles due to the action of the fixative, and reenforced by the deposition of silver or osmium. Nevertheless, it is the 'contents' which stain in neutral red, and silver or osmium are held to behave in a manner similar to neutral red (Parat and Painlevé, '25 f). Thus far the views of these three schools have much in common. Parat, however, goes a step farther and finds that networks are often due not to mere internal blackening of the vacuoles or their coalesced aggregates, but to the deposition of silver or osmium around or between the vacuolar walls. It will thus be apparent that the vacuolar theories include many shades of opinion, with, however, a common basis of belief in the view that the Golgi apparatus is really represented by the contents of a vacuolar ensemble.

that has objective existence is the watery vacuome, and this constitutes the real Golgi apparatus. The vacuome is the Golgi apparatus, and the so-called Golgi material is only an artifact—a myth, which has no material basis whatever.

Having thus bowed the classical Golgi apparatus out of the cell, and transferred the name to the vacuoles, Parat considers the vacuome theory as completely established from a morphological point of view, leaving for consideration only its mode of functioning. As already pointed out, secretory granules are essentially vacuoles within which, perhaps by processes of condensation, the secretory granules proper are formed. By Parat these vacuoles are looked upon as the vacuome of the secretory cell, and as such homologous with the vacuoles in any other cell whether secretory or not. In secretory cells the Golgi apparatus (i.e., vacuome) has thus a secretory function, though on a basis quite different from that postulated in the recent theories of secretion based on the classical Golgi apparatus. What the vacuome is doing in types of cells other than secretory, Parat does not elucidate, and in this respect his theory cannot honestly be criticised, since the same problem has proved difficult in the case of the classical Golgi apparatus.

To recapitulate, the vacuome theory holds that all cells contain two types, and two only, of fundamental structural elements—the chondriome and vacuome. The vacuome consists of an aggregation of spherical vacuoles with a watery content, specifically staining in neutral red. These vacuoles constitute the Golgi apparatus. The classical Golgi material in animal cells, blackened by osmic acid or silver nitrate, is an artifact pure and simple, due to the deposition of the impregnating medium within, on the surface of, or between, the vacuoles. Within the vacuoles secretory granules are formed by concentration of the vacuolar contents.

An obvious criticism of this theory suggests itself at once. In the pulmonate molluscs, e.g., *Helix*, the classical Golgi apparatus is supposed to consist of a number of rod-shaped bodies applied to the surface of an idiosome, the whole being associated with the manufacture of the acrosomal granule.

These bodies, readily visible *intra vitam*, are obviously not vacuoles and are stained by Janus green. It is thus neither possible to interpret them as a part of the vacuome nor to dismiss them as non-existent in the living cell. This difficulty is circumvented by Parat, first, through the fact that in the center of the Golgi apparatus-idiosome mass there occurs a group of vacuoles which readily stain in neutral red, as had been previously shown by Karpova ('25). This is the vacuome proper. In the second place, according to Parat, the so-called Golgi rodlets are not Golgi material at all, but are merely a modified part of the chondriome, which Parat terms *lepic hondriosomes* or *lepidosomes*. The fact that they stain with Janus green accords well with this interpretation. The conception of chondriome and vacuome as the only primary constituents of the cytoplasm is thus supposed to be saved.

This will, I think, suffice to give a fair impression of the general idea involved in the vacuome theory, without adding further specific examples of Parat's results, which are remarkably uniform in content. It remains to undertake a critique of the theory as a whole. Thus far pointed criticism of the theory has been made only by Avel ('25 a, b, c), who raises two fundamental objections: first, that the presence of a permanent system of true vacuoles in all animal cells is unproved, and, second, that neutral red is in any event not specific for vacuoles. These objections strike at the very foundation of the theory, but actually are difficult either to sustain or to disprove. The exact application of neutral red to living animal cells is relatively a new thing, and it is difficult to be sure that no vacuoles occur in cells where they are not demonstrated by a particular trial. Furthermore, the fact remains that positive results are universally obtained in plant cells and quite frequently in animal cells, and there is at least a presumption in Parat's favor that all cells do possess vacuoles which may conceivably be fundamentally similar. If such a view could be supported by extended examination of animal cells, it would in itself be a matter of

the most extraordinary interest. For it is exactly the universal occurrence of vacuoles in plant cells which just now confuses the comparison of animal and plant cytoplasm. In other words, I believe that in focusing his attention on the supposed relation between Golgi apparatus and vacuoles, Parat has missed exactly what might prove to be the most interesting feature of his studies. As I hope presently to show, the conception of the vacuome as the Golgi apparatus is in itself out of the question on the basis of current evidence, but the problems presented by the vacuome as such are in themselves of far-reaching interest. Thus we should like to know whether all animal cells, critically examined, do possess vacuoles stainable with neutral red. We should like some method of staining these vacuoles more or less specifically in permanent preparations. We need very much to know whether plant and animal vacuoles are homologous, and where, if at all, the contractile vacuoles fit into the scheme of the vacuome as a whole. Finally, the relation existing between the vacuome and the classical Golgi apparatus remains to be elucidated, particularly in plants.

Passing from these fundamental characters of the vacuome, which are in any event of the greatest interest, there remains the question as to the equivalence of Golgi apparatus and vacuome. The only point to be settled is whether there is such a thing as the classical Golgi apparatus—a material, that is, which has a real identity quite apart from the vacuome proper. Parat holds that the classical Golgi substance is non-existent and that no lipoids are associated with the vacuoles.

The question as to the chemical nature of the classical Golgi material has long been in an unsatisfactory state, due to the fact that the well-known fat tests do not usually work, and the blackening with osmic acid is not perhaps sufficiently specific to be acceptable in case of doubt. In two cases, however, fat tests have been applied to the Golgi material with positive results—Ciaccio's test by Weiner ('26) on the intestinal cells of the frog and Kingsbury's test by Cowdry

('11) on nerve cells of the pigeon. Just how much these results may actually prove it is hard to say, but at all events they squarely contradict Parat's statements and place the burden of further proof on his shoulders. So far as fat tests have succeeded, they indicate the presence of Golgi substance as a material reality.

As to the development of the Golgi reticulum, particularly in gland cells, through the fortuitous precipitation of the impregnating medium, as argued by Parat, the results of recent comparative studies on secretion offer interesting commentaries. In the first place, Avel ('25 b) finds that osmic acid does not cause deposits within animal-cell vacuoles, as should occasionally occur in accordance with one of the three possibilities claimed by Parat. This is abundantly corroborated by all workers on gland cells, and where such blackening does occur, as, for example, in lipoid gland cells (Bowen, '26 f), there is not the slightest evidence that this blackening has anything to do with the production of a false Golgi apparatus. On the other hand, in lipoidal gland cells of different kinds, the Golgi material appears in various forms, always characteristic for a given cell type, but widely different, for example, in the rabbit's inguinal gland and the oil gland of the fowl.⁵ Why do the salivary gland cells of an invertebrate, *Limax*, present their Golgi apparatus in the form of isolated bodies, while in vertebrates the same material is a plate- or network (Bowen, '26 e)? Innumerable

⁵It is a point of some interest to note that, with the usual osmic-acid methods for demonstrating the Golgi apparatus, the general results on the Golgi material in lipoidal glands are like those obtained in mucous and serous gland cells (compare Bowen, '26 e and f). In other words, one would be led to conclude that the structural features and the mechanisms at work are the same in all kinds of gland cells. Nevertheless, Parat and Painlevé ('25 f) state that fatty or lipoidal granules are not contained within vacuoles, and in the majority of cases are not stainable with neutral red. It will be recalled that Altmann's old studies on lipoidal granules demonstrated a structure morphologically like that of secretory granules of other types. The attempt to set apart the lipoidal secretory granules in a special class exempt from the application of the vacuome theory, when, as a matter of fact, the Golgi-apparatus methods show conditions similar to other types of gland cells, would seem to be another very serious objection to the vacuome theory as a whole.

examples of the definite behavior of the Golgi material might be marshaled, but this would be needless repetition. The fact seems undeniable that the behavior of the Golgi apparatus can be accounted for only on the basis of the presence of a real substance—the known conditions being inexplicable on the basis of a mere chance deposition between the walls of the secretory vacuoles. But finally, and most serious of all, is the fact that in the promeristem cells in plants spherical vacuoles occur packed closely together as in an animal gland cell. But upon impregnation these vacuoles may blacken without the remotest resemblance to a Golgi network, and between the vacuoles no artifacts ever produce the magnificent structures revealed by first-class preparations of an animal secretory cell. And all this is still valid in spite of the fact that, as Parat also maintains, the Golgi apparatus actually is frequently distorted into appearances which it obviously could not have had in life.

The explanation of the condition in the spermatid offered by Parat seems to me to be based on very dangerous ground. In effect he relies upon the fact that no one has yet followed the classical Golgi material from embryonic cells into the male germ cells. Nevertheless, Hirschler's ('18) work on the molluscan embryo and the obvious similarity between the Golgi substance in insect germ cells (Bowen, '20) and in nerve cells (Bialkowska and Kulikowska, '12) certainly establish a probability that, whatever this substance is, it is homologous throughout the range of animal cells, and is present in the male germ cells of *Helix* just as in all other cells.

Finally, it now seems certain that the conditions in the plant cell, upon the supposedly accurate description of which Parat and his supporters have implicitly relied, are more complicated than had been believed. The osmiophilic platelets introduce into the picture a structure of fundamental character, which has not been seen to stain *intra vitam*, in either Janus green or neutral red. Obviously, something has been overlooked, and it is exactly this material which seems to be the homologue of the classical Golgi substance in

animal cells. I do not care now to press this point too hard, for the reason that the facts are so new that they have not yet been adequately examined by other workers, but the implications are obvious. With the appearance of the osmiophilic platelets on the scene, the simple and attractive theories of cytoplasmic structure erected by Guilliermond and Parat must fall to the ground, without, however, in any way prejudicing the many interesting and important facts upon the discovery of which the vacuome theory so largely depends.

My own conclusion is that the classical Golgi apparatus is in itself a reality, present in various shapes in all cells, and that in many, perhaps all, cells there exists also a vacuolar system. Golgi substance and vacuome each have a real existence, although as in animal secretory cells and spermatids a most intimate relation may exist between them (Bowen, '26 g). To what extent the vacuoles in different kinds of cells, more particularly those of plant or animal origin considered as groups, may be homologous must for the present be left undecided. Finally, it seems only fair that before criticising this critique, other workers should first present evidence affecting the validity of my description of the osmiophilic platelets, since upon these so much of the argument ultimately depends.

HOMOLOGIES OF PLANT AND ANIMAL CYTOPLASM

In conclusion, it seems necessary to return briefly to the eternally perplexing problems involved in the comparison of animal and plant cytoplasmic structure. I want chiefly to point out that the crux of the situation depends upon the fact that (leaving the central bodies out of account) plant cells seem to possess in their cytoplasm four independent and universal formed elements, while animal cells have only two (excluding for a moment the animal vacuoles). Admitting that the pseudochondriome of the plant cell is equivalent to the animal chondriome, the plastidome remains unaccounted for. Similarly, if the osmiophilic platelets of the plant cell are equivalent to the animal Golgi apparatus, the vacuome remains unexplained.

In each case two alternative explanations seem possible. In the case of the plastidome, we may be dealing either with a specially differentiated part of the chondriome, as Guilliermond and others have held, or with two quite different elements, of which the plastidome is without representation in animal cells. In this case it is still possible that the plastidome is phylogenetically related to the chondriome—a matter which in itself would be difficult, if not impossible, to prove either one way or the other. Perhaps some combination of the two alternatives may be the correct explanation, the plastidome being under normal conditions independent and self-perpetuating, but still related to the chondriome through the possibility of its restoration at the expense of chondriosomes in cases of emergency. This whole question is one upon which much critical work remains to be done.

With respect to the plant vacuome and the animal secretory vacuoles, the simplest explanation would be that they are in some way homologous structures, morphologically independent of the Golgi material. This possibility is so contrary to the conditions apparently existing in animal cells, that it would seem easier to consider the vacuoles as a secondary product of the activity of the Golgi apparatus—as has actually been done in recent theories of secretion in animal cells. Unfortunately, there is no indication that the plant vacuome has any relation to the Golgi apparatus (osmiophilic platelets). Possibly the vacuole walls in plants may represent Golgi material, or the vacuoles may be in some way dependent upon the activity of the osmiophilic platelets. Neither of these possibilities seems, in the light of present scanty knowledge, very probable. The fact that vacuolar primordia are always present in the youngest plant cells suggests a certain degree of autonomy and causes one to wonder whether their walls may once have been related to Golgi material, in the same way that the plastids may once have been related to chondriosomes. Finally, it is possible that the vacuome in plant cells is something quite different from such vacuoles as are known to occur in animal cells, e.g., secretory vacuoles,

but this conception leaves the whole question of the vacuole as a center of secretory processes in the plant cell somewhat out of joint with our recent theories of secretion in animal cells.

If this sounds like confusion, we may console ourselves with the fact that recent cytological researches should have carried us so far that all these questions can now be intelligently raised. The old puzzle of protoplasmic structure is still with us, but it is a hopeful sign of progress that it is at last beginning to assume a shape in which definite problems emerge, for which we may hope to gain satisfactory answers.

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A METHOD FOR THE DEMONSTRATION OF THE INTRACELLULAR SECRETION CANALICULI OF THE PARIETAL CELLS OF MAMMALS

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Since the discovery of the intracellular secretion canaliculi in the parietal cells of mammals by Müller ('92) histologists have depended for the most part on the precipitation silver methods for the demonstration of these structures. Preparations made by these methods, unfortunately, are of rather indifferent value from the cytological standpoint, and the study of the changes in the contour and content of the canals associated with functional changes in the cells awaits a method which, while effective from the standpoint of the canals, is also without objection from that of cytological fixation.

At the present time the most satisfactory and complete method of demonstrating the canals and their contents is that discovered by Harvey and Bensley ('12), who found that a number of vital dyes injected intravascularly in very dilute solution in isotonic salt solutions stained all of the secretion within the canaliculi of the parietal cells, and so permitted the observer to view even the finest ramifications. Of these dyes employed by Harvey and Bensley, the most readily obtainable is neutral red, but similar results were obtained by many dyes belonging to the Nile-blue series of compounds.

Unfortunately, the beautiful preparations made by the method of Harvey and Bensley have proved incapable of preservation, and, while of great service in studying the secretion in the surviving tissue, do not meet fully the needs of cytological research.

In the course of an investigation on the functional changes in the cells of the gastric glands during rest and activity carried on in conjunction with R. K. S. Lim, I found it possible by a new method to stain the secretion in the canaliculi of the parietal cells and in the lumen of the fundus gland, and at the same time to stain the mitochondria and secretion granules in the cells differentially. In the paper of Lim and Ma ('26) these results are referred to as stainable coagula in the canaliculi, but the staining technique is not described in detail.

FIXATION

The tissues, removed with as little delay as possible from the stomach of an animal (rabbit) killed by a blow on the head, are placed in Regaud's solution containing eight parts of a 3 per cent solution of potassium bichromate and two parts of neutralized formalin. In this solution they remain for three days. The solution is changed daily. On the fourth day the tissues are transferred to a 3 per cent solution of potassium bichromate, in which they remain for six days more, the solution being changed every other day. They are then washed, dehydrated, embedded in paraffin according to the usual methods, and sectioned. The sections should not exceed 3μ in thickness.

SIMPLE METHOD

Preliminary to the staining, the sections are passed through xylol and graded alcohols to distilled water, then treated with 1 per cent permanganate of potassium for thirty seconds, 5 per cent oxalic acid for thirty seconds, then transferred to distilled water.

The sections are then stained for fifteen to twenty minutes in Mallory's connective-tissue stain containing aniline blue and orange G. They are then washed, dehydrated, cleared, and mounted in balsam. The secretion in the canaliculi of the parietal cells is stained blue, while the granular cytoplasm is stained yellow. In some cases the secretion exhibits itself

as an irregular blue-stained smear on the sides of clear open canaliculi. This appearance is obviously due to shrinkage of the secretion in fixation. In the lumina of the fundus glands in activity may be seen a coagulum which is sometimes blue, sometimes yellow, which is obviously a mixture of the products of the parietal and body chief cells, respectively.

DOUBLE STAIN

After treatment with oxalic acid and permanganate as above, the sections are stained warm in Altmann's anilin acid fuchsin solution for a period of two to three minutes. They are then washed thoroughly in distilled water and placed for one minute in a 1 per cent solution of phosphomolybdic acid. They are then washed again in distilled water, and stained as above in Mallory's blue solution for connective tissue.

By this method the granules and mitochondria of the chief cells are stained red, the nuclei blue, the granules of the parietal cells red or yellow, nuclei blue, and secretion in the intracellular secretion canaliculi blue.

The success of this method depends primarily on the successful precipitation of the intracellular secretion and that in the gland lumina by means of the Regaud's solution. It should prove useful not only for studying the changes in amount of this secretion in different functional phases, but also for preparing the gastric glands for microchemical examination of their secretion.

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POLYOVULAR FOLLICLES AND POLYNUCLEAR OVA IN THE MOUSE

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The recent discussion of Hartman ('26), regarding polynuclear ova and polyovular follicles, emphasizes the common occurrence of such follicles and ova in the majority of animals on which reports have been made.

Hartman's thorough review of the extensive literature makes it necessary to refer only to those papers which apply directly to this study. There is only a single report—Chappellier ('09)—regarding non-atretic polyovular follicles, and one (Blanc, quoted by Hartman) mentioning a polynuclear ovum, in the mouse. Kingery ('14) mentions polynuclear ova, but it is clear that he regarded these as being atretic.

The data presented here are the result of a study of one hundred ovaries of the mouse, representing sixty-four adult animals, a single ovary having been removed from one group. The collection of these data on the occurrence of polynuclear ova and polyovular follicles was incidental to a study of follicular atresia (Engle, '27).

Polyovular follicles are not so abundant in the mouse as they are in the opossum. They were found in only twelve ovaries in the series of one hundred of the former, while Hartman reported one or more such follicles in all but twelve of 150 of the latter. A polynuclear ovum was found but twice in this series. It is necessary to exclude atretic ova with fragmented nuclei, which often give the appearance of a polynuclear condition, but which are secondary in origin and not true polynuclears. These two polynuclear ova, one with two and one with three nuclei, were clearly not the result of atresia.

Two triovular follicles of medium size ($400\ \mu$ each) with large antra folliculi and average sized ova were found. In one of these, pycnotic cells characteristic of degeneration were abundant in the antrum (Engle, '27). For comparison it may be stated that mature follicles in this material are about $750\ \mu$ in diameter.

Sixteen biovular follicles occurred in this series, in seven of which the antrum folliculi had not yet formed. One of these latter showed two cells in a common zona pellicula, and hence resulted from cell division. The blastomeres were of equal size, and the nuclei were in a resting stage. This is similar to the follicle containing four blastomeres, two of which possessed spindles, reported previously (op.cit.).

A single follicle showed ova of unequal size. It contained one ovum of $21\ \mu$ and one of $40\ \mu$. The remaining five instances contained ova of equal size, separated by two or more layers of granulosa cells.

Nine of the biovular follicles were small, under $250\ \mu$ in greatest diameter, but a small antrum follicle had formed. In six of these the ova showed considerable disparity in size, the smaller one usually being about one-half the size of the larger. In two cases the smaller ovum contained a crystalloid body and a fragmenting nucleus, and hence is to be considered as atretic.

The ova in these cases were surrounded by an individual granulosa, and were not in contact. In most of them the antrum had formed between the ova.

The number of polyovular follicles is too small to be of significance in determining their origin, but the idea expressed by Hartman, that they represent imperfectly separated portions of the egg tube, seems to offer fewer difficulties than other current theories. Those follicles which contain ova of unequal size represent unequal rates of growth, with the probability that not more than one ovum would reach maturity.

Thirteen anovular follicles occurred in this series. All of these were large follicles, in which the ovum had undergone

cytolysis and in which the corpus luteum atreticum had not formed.

This interpretation is derived from a study of the transitional stages which occur. In the majority of the larger atretic follicles a corpus luteum atreticum, often with retained ovum, is formed. In follicles in which this has not occurred, a greatly reduced granulosa borders the theca interna. The naked ovum, unsupported in the antrum, is in varying stages of cytolysis. These follicles with a naked ovum have much the same appearance as the anovular follicles, except that no trace of the ovum is found in the latter.

Small and medium-sized anovular follicles, as reported by League and Hartman ('25), were not found in the mouse.

SUMMARY

In a series of one hundred mouse ovaries, only two polynuclear ova were found in which the polynuclear condition was thought not to be due to degenerative changes within the ovum.

Two triovular follicles and sixteen biovular follicles were present. Two of these biovular follicles were in one ovary and three in each of two ovaries.

The thirteen anovular follicles were cases of advanced atresia in which the destruction of the ovum had preceded the disintegration or replacement of the granulosa.

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A GROUP OF SMALL CELLS IN THE HYPOGLOSSAL NUCLEUS OF MAN

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TWO FIGURES

Microscopic examination of consecutive sections of the human brain stem shows an oval group of small cells lying within the hypoglossal nucleus or close to its medial border, at about the level of the junction of its caudal and middle thirds. Obersteiner ('12) mentions that occasionally a small spherical body containing cells of this type may be seen embedded in various motor nuclei, particularly in the hypoglossal nucleus. McLean ('26) found that the cells of the third, fourth, and sixth cranial nuclei of the dog and cat could be separated into two distinct sizes, in general diffusely intermingled throughout the nuclei. Other than this no mention is made of such cells in the literature.

It was first thought that these cells might be those of Roller's nucleus. However, this latter group of cells is found ventromesial to the cephalic third of the nucleus of the XII nerve. Moreover, Roller's nucleus could be identified in the sections studied in addition to the small group of cells embedded in the hypoglossal nucleus.

For this reason an investigation was undertaken in order to determine the position, frequency, and morphology of this group of cells. Seven human brain stems of various ages, from that of a fetus of thirty-three weeks to adult, were examined. Six were stained by the Weigert-Pal method, the other with thionin, for the purpose of studying cell structure. Drawings of each section were made with the aid of a camera lucida, so that reconstructions might later be possible and the dimensions of the group thus determined.

The nucleus was found constantly present in all seven brain stems. It was roughly ellipsoidal in shape and bilateral, occurring at about the level of the caudal and middle thirds of the hypoglossal nucleus. It was usually found inside the hypoglossal nucleus, although in three of the stems it was seen lying just medial to the inner border. Following is a brief summary of its form and location in each of the brain stems studied.

Brain I. Human adult brain stem, stained by the Weigert-Pal method and counterstained with carmine. The sections were 40 μ thick and cut transversely. It was in this brain that the nucleus was first seen, occurring as an area of small cells, well marked out by a thick rim of fibers. A reconstruction of the nuclear masses in this brain has been made by Weed ('14), so that an excellent means of orientation was available for the present study. On this account, special attention was afforded the location and shape of the nucleus in this material.

As the sections of this brain are examined, proceeding cephalad, the cell group is first seen on the left side of the brain at about the termination of the caudal third of the hypoglossal nucleus. It may be followed through succeeding sections, growing gradually larger and then diminishing in size. A nucleus symmetrical with this appears on the right in a slightly more rostral location, so that the two overlap for approximately a third of their length. In cross-section, the nucleus appears as an oval group of very small cells lying well within the medial border of the hypoglossal nucleus. Very few fibers can be seen among the cells, but the cell group is entirely surrounded by a dense band of fibers which seem to radiate into the hypoglossal nucleus and particularly toward the hypoglossal nerve. A photomicrograph of a typical cross-section of the nucleus is reproduced herewith (fig. 1). This shows the cell group, well defined by a thick circumferential layer of fibers, lying embedded in the right hypoglossal nucleus. The base of the median sulcus can just be seen. Fibers may be traced from the ventromedial border

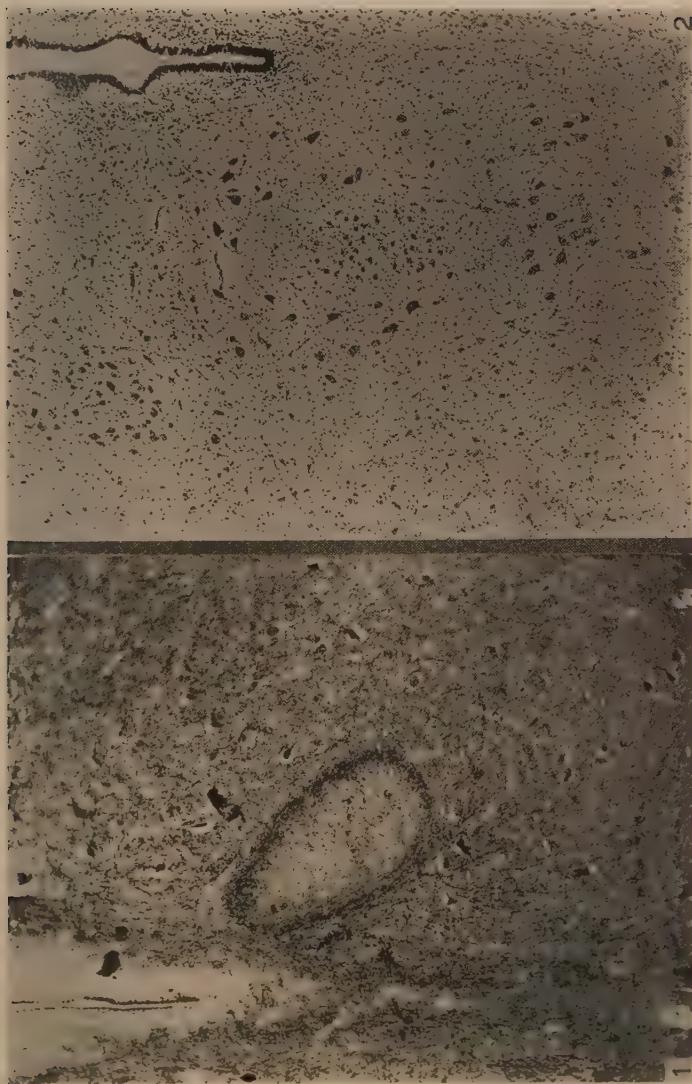


Fig. 1 Photomicrograph of a transverse section of a human adult brain (brain I) stained by Weigert-Pal method. A small group of cells surrounded by a thick layer of fibers is seen embedded in the right hypoglossal nucleus. The median sulcus is seen in the upper left-hand corner. $\times 55$.

Fig. 2 Photomicrograph of a transverse section of a brain of a human fetus (brain II) stained with thionin. A group of small cells is embedded in the center of the left hypoglossal nucleus. Cells of the dorsal motor nucleus of the vagus nerve are seen in the upper left-hand corner; the median sulcus, in the upper right. $\times 55$.

of this cell group, passing ventrally through the hypoglossal nucleus. On the right, the cell group measures $0.56 \times 0.63 \times 0.37$ mm., lying slightly more rostrally than that on the left. The dimensions of the left nucleus are $0.56 \times 0.46 \times 0.26$ mm.

Brain II. This brain was obtained through the courtesy of the Department of Embryology of the Carnegie Institution of Washington. It was taken from a human fetus of approximately thirty-three weeks. The brain was fixed in formalin, mounted in celloidin, and stained with thionin. The sections were cut 25μ thick. This brain was especially prepared so that the cells of this small nucleus might be studied.

In this specimen the nucleus itself appears in a slightly more caudal position on the left than on the right. In transverse sections it is seen in the center of the hypoglossal nucleus. Its rostrocaudal location is again at about the junction of the middle and caudal thirds of the hypoglossal nucleus; its dimensions are $0.23 \times 0.31 \times 0.19$ mm. on the right and $0.20 \times 0.35 \times 0.19$ mm. on the left.

Since the staining is with thionin, no fibers can be seen to mark out the location of this nucleus in the sections. However, it appears quite definitely as an oval group of very small cells in the center of the large cells of the hypoglossal nucleus. A photomicrograph of a typical section of this series is here reproduced (fig. 2), and gives some idea of the comparative size of the cells and the size and shape of the nucleus.

The cells are about one-half or one-third the size of the motor cells of the hypoglossal nucleus and are slightly smaller than the autonomic cells of the dorsal motor nucleus of the vagus nerve. They are essentially oval in shape, showing typical axone hillocks. The cytoplasm contains a supply of dust-like tigroid element whose distribution is quite uniform, except that large granules are collected along the edge of the cell, particularly at the hillock bases. The nuclei of these cells appear as clear spherical vesicles possessing distinct nuclear membranes and lying usually in the centers of the cells. Within the nuclei are found single, small, round, deeply

staining nucleoli, each of which contains a still smaller chromophobic nucleololus. The remaining interior of the nucleus shows a linin framework and some chromatin.

Brains III and IV. These two brain-stems were taken from newborn human infants and stained by the Weigert-Pal method. They were used by Sabin ('01) for the reconstruction of a model of the medulla and midbrain, so that, again, a reference is available for orientation. The sections were cut at 70μ , but both series are composed of every alternate section, so that each slide represents two sections, or 140μ .

The small oval group of cells seems to occur in each brain at about the same level on both sides—or at the junction of the middle and caudal thirds of the hypoglossal nucleus. On each side the cell group is placed just medial to the hypoglossal nucleus of its corresponding half and lateral to the median line. On account of the small size of this cell-group in the newborn and the great distance between sections, it is difficult to reconstruct these series in three dimensions. However, the general ellipsoidal shape is apparent, the dimensions in brain III being $0.28 \times 0.26 \times 0.17$ mm. on the right and $0.28 \times 0.18 \times 0.14$ mm. on the left. In brain IV the nucleus measures approximately $0.28 \times 0.29 \times 0.17$ mm. on the right and $0.28 \times 0.29 \times 0.18$ mm. on the left.

Brain V. This is a human adult brain; fixed in formalin, embedded in celloidin, and cut transversely into sections 40μ thick. The Weigert-Pal method of staining was employed with carmine counterstain.

The nucleus appears as an oval group of small cells well surrounded by a thick layer of fibers and lying embedded in the hypoglossal nucleus. On the right it measures $0.48 \times 0.43 \times 0.29$ mm., and lies slightly more rostrally than on the left. Its dimensions on the left are $0.52 \times 0.48 \times 0.31$ mm.

Brain VI. No records are available concerning this brain stem, although it might be judged from its size to be that of a newborn. The sections have been mounted in celloidin and stained by the Weigert-Pal method, counterstained with carmine. The nucleus on the left is distorted somewhat by an

artifact, but it appears as an oval group of very small cells in the center of the hypoglossal nucleus. On the right the cell group is very distinct and similarly placed. It, too, follows the ellipsoidal shape common to other specimens and is surrounded by the characteristic dense area of fibers. The dimensions along the dorsoventral axis and the lateral axis are 0.29 and 0.18 mm., respectively.

Brain VII. This is an adult brain from which only a few sections are available. This specimen is stained with the Weigert technique. A nucleus corresponding to the one being described is found on the left side of the brain, just medial to the hypoglossal nucleus. The few sections available preclude the determination of the rostrocaudal dimension and the location of the nucleus on the right. The group on the left measures in transverse section 0.42×0.25 mm.

TABLE 1

Giving the dimensions of the nucleus in the various brains studied

BRAIN	AGE	SIDE	DIMENSIONS		
			Rostrocaudal	Dorsoventral	Right-left
			mm.	mm.	mm.
I	Adult	Right	0.56	0.63	0.37
		Left	0.56	0.46	0.26
II	Thirty-three-week fetus	Right	0.23	0.31	0.19
		Left	0.20	0.35	0.19
III	Newborn	Right	0.28	0.26	0.17
		Left	0.28	0.18	0.14
IV	Newborn	Right	0.28	0.29	0.17
		Left	0.28	0.29	0.18
V	Adult	Right	0.48	0.43	0.29
		Left	0.52	0.48	0.31
VI		Right		0.29	0.18
		Left	Distorted by artifact		
VII	Adult	Left		0.42	0.25

DISCUSSION

It appears from a study of these brain stems that this small group of cells is a definite and constant structure in the human brain. It is essentially ellipsoidal in shape, lying quite uniformly at the level of the junction of the middle and caudal thirds of the hypoglossal nucleus. In transverse sections it is seen to lie usually within the hypoglossal nucleus, but at times just medial to it and closely in contact with its inner border. It is bilateral; in the majority of the brains studied it appears in a slightly more caudal position on the left than on the right. Since the size of the nucleus is so small, this apparent asymmetry is probably merely a coincidence. The accompanying table gives the dimensions of the nucleus in the various brains studied. A surprising uniformity is displayed, especially if the ages of the various brains are considered. As an average, it would seem to measure in the adult brain 0.50 mm. in rostrocaudal length, 0.45 mm. in the dorsoventral axis, and 0.30 mm. from left to right.

Its structure is quite uniform. It is made up of very small cells surrounded by a distinctive dense layer of fibers which seem to radiate toward the ventral side of the hypoglossal nucleus and mingle with the hypoglossal fibers.

It was intended, if possible, to study the function of this group of cells. However, on examination of the brains of cats and rabbits, no such nucleus could be located, so that any work on the common experimental animals was precluded. A study of this nucleus in man from a pathological aspect is obviously quite impractical. It is therefore possible only to hypothesize concerning its function.

This cell group might be considered as a part of some other nucleus. Evidence that it is not Roller's nucleus has already been given. However, it might be a portion of the *nu. funiculi teretis*. Spiegel ('17) has called attention to the fact that one part of this nucleus lies along the *raphé*, between the two hypoglossal nuclei, in a somewhat more distal position than its other more easily recognized portion. Jacobsohn ('19) designates this part as the *nu. paramedianus dorsalis*, giving

the designation *nu. funiculi teretis* to an entirely different cell group which is designated as the *nu. prepositus XII* by Winkler ('18) and Villiger ('25), and as a portion of the *nu. intercalatus* by Ziehen ('26), Streeter ('02), and Weed ('14). There is, however, some evidence that this cell group is not identical with the *nu. funiculi teretis*, since, in addition to the cell group under discussion, another nucleus, lying more closely against the *raphé* and corresponding to the distal portion of the *nu. funiculi teretis*, may be demonstrated. Moreover, the manner in which the fibers from this cell group seem to mingle with those which comprise the hypoglossal nerve would make it seem probable that its function has to do with the hypoglossal nucleus, rather than with the medullary striae, with which the *nu. funiculi teretis* is generally supposed to be linked.

The cells, therefore, may contribute fibers to the *n. hypoglossus*: this is strongly suggested by the position of the nucleus. It is possible with this hypothesis to speculate concerning the type of impulse which the composing cells mediate. McLean ('26) has suggested that the small cells found in the motor nuclei of the oculomotor, trochlear, and abducens nerves are sensory, carrying proprioceptive impulses from the extraocular muscles of the eye. Langworthy ('24) reported that typical sensory nerve endings were present in the tongue musculature. Moreover, it has been shown by Streeter ('02) that the *n. hypoglossus* has been derived phylogenetically from a fusion of three or four segmental nerves. Sensory ganglia are still present upon the hypoglossal nerve of some vertebrates; Langworthy ('24) mentions that such a ganglion is often found in the cat and that three ganglia were found upon one nerve in the pig. Such a collection of ganglion cells, rare in adult man, has been described by Froriep ('82) in a human fetus; this ganglion is often called by his name. It is possible, therefore, that, in connection with the third, fourth, sixth, and twelfth nerves, primary sensory neurones mediating proprioceptive impulses may have been included in the brain stem and have been incorporated with the motor

nuclei of these nerves; such sensory cells may be recognized by their smaller size.

But there seems to be one serious objection to the acceptance of this thesis. It is now well established that primary sensory cells whose nerve fibers carry afferent impulses from the muscles of mastication are found in the midbrain; their fibers join the motor division of the n. trigeminus (Johnston, '09, and Allen, '18). These cells are typical sensory cells having the size, contour, and internal structure of those found in spinal and cranial ganglia. It is difficult to imagine that other primary sensory neurones included in the brain stem would deviate so markedly in their general appearance.

For the components of the nucleus under consideration are not typical sensory cells, but, according to the histological classification of Malone ('13), they would appear to be of an autonomic nature. For this reason it would seem probable that this nucleus contributes autonomic fibers to the hypoglossal nerve. That such fibers are present has long been known, although their source has not as yet been established. A portion of them reach the n. hypoglossus from the superior cervical ganglion. There is some evidence (van der Sprenkel, '24) that Roller's nucleus also contributes a supply, although the exact function of this nucleus has not been determined.

A consideration, therefore, of the evidence supporting the three theories which have been given concerning the function of this nucleus would suggest that most probably it contributes autonomic fibers, possibly of vasomotor function, to the hypoglossal nerve.

SUMMARY

A description is given of a group of small cells, embedded in the hypoglossal nucleus of man at about the level of the junction of its caudal and middle thirds. It is ellipsoidal in shape, composed of very small cells, and surrounded by a dense layer of fibers. In the human adult it measures approximately $0.50 \times 0.45 \times 0.30$ mm.

Three hypotheses concerning its function are considered. It may be a portion of the nu. funiculi teretis. It may be made up of the cells of primary sensory neurones innervating the tongue musculature, and thus be proprioceptive in character. Or, more probably, it may be a source of autonomic fibers to the tongue.

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CROSS-SECTION CONTAINER OF ALUMINUM

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FIVE FIGURES

Anatomists have recognized the fact that the teaching of cross-section anatomy presents a number of difficulties unless the sections of the body can be permanently mounted. Students dislike the handling of formalin material, and consequently prints are studied rather than the actual sections. Preparations are easily torn and, if exposed to room temperature for several hours in succession, dry out and become discolored, making them unsuitable for future use. This necessitates frequent renewal of cross-section material, which is laborious and expensive, particularly for those medical schools where cadavers are scarce. The various parts of a section are best exposed when submerged in a liquid, and this is done with difficulty unless suitable containers are available.

To overcome these handicaps and many others too obvious to deserve mention here, various kinds of containers have come into vogue. As far as the writer has been able to ascertain, most of those containers are made of glass or some other fragile material. Of necessity they are massive and readily breakable, which makes them only suitable for museum exhibitions, but impractical for students' use in the laboratory. It seems that a desirable container for cross-section anatomy as well as for museum use must first of all be of such structure that it can be passed from student to student with the minimum danger of breakage. It must be as small as possible and as light in weight as it can be made. It must permit of the filling and refilling with fluid and the mounting and re-mounting of material. It must be neat in appearance and of

moderate cost. With those points in mind, the writer has designed and constructed a container which is described in the following pages.

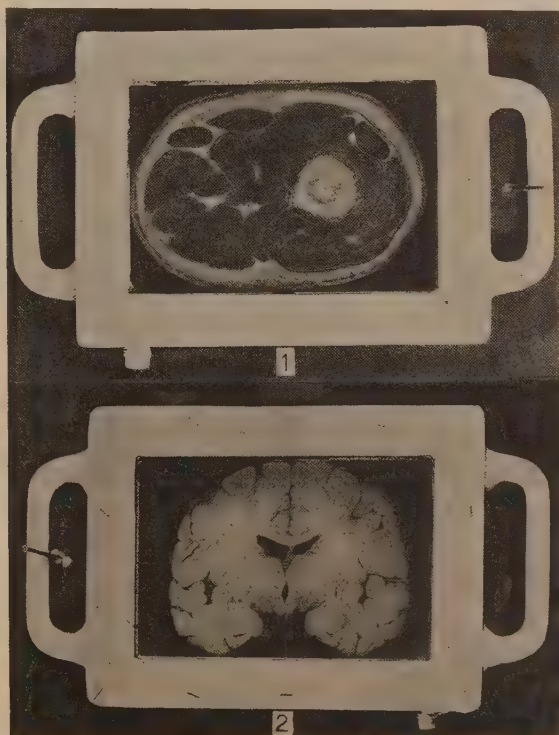
CONSTRUCTION OF CONTAINER FOR CROSS-SECTIONS OF THE BODY

Aluminum was chosen as the material for the manufacture of the frame, as it is light in weight, substantial in texture, of moderate cost, and permits of an attractive finish. To economize space and add to the smoothness of handling, it is made rectangular in shape with rounded edges and provided with a substantial handle at each end. The frame consists of three pieces, the body and two cover-plates (figs. 1 to 5). The body is a hollow rectangle, the rim of which is 1 inch in width and $1\frac{1}{4}$ inches in thickness. On each side of the rim next the cavity there is a depression $\frac{1}{8}$ inch deep to receive the glass plates and cement. The bottom of each depression is $\frac{1}{2}$ inch in width, and between them is a ledge 1 inch thick. The faces of this ledge make the four walls of the chamber. The ledges should be perfectly straight and level throughout, in order to support the glass plates properly. The ledge is furrowed for a cement groove $\frac{3}{16}$ inch wide and $\frac{1}{16}$ inch deep (fig. 4). The filling hole is drilled entirely through the body and reaches the chamber of the container as close to one corner as it can be made. The purpose of this is to eliminate air bubbles while filling the chamber. The hole is capped with a nickel-plated brass screw plug. The rim of the body is drilled and tapped for $\frac{3}{16}$ -inch screws, which are placed approximately 2 inches apart. The exposed surfaces of the body as well as the handles are machined and nicely polished.

The cover-plates are made of cast aluminum and measure 1 inch in width and $\frac{1}{8}$ inch in thickness. They are drilled and countersunk for flat-head machine screws. One plate is screwed to each side of the body and serves to hold the glass plate in place. The edges are rounded and the exposed surfaces polished (fig. 5).

The containers have been designed for double-strength window glass. Experience, however, has proved that single-

strength glass is more satisfactory. It should be relatively straight, of even thickness, and clear. The glass should be cut slightly smaller than the depression in the frame to allow expansion. Of course, it must be perfectly clean before it is used for mounting.



Figures 1 and 2

Only three sizes of containers for sections of the body have been manufactured thus far. The largest type, designed to hold sections through the shoulders, measures 9 inches wide and 18 inches long. The medium-size ones measure 9 inches wide and 14 inches long, and are suitable for sections of the head, neck, thorax, abdomen, and upper part of the thigh.

The smallest size, 6 inches wide and 8 inches long, is large enough for sections of upper and lower extremities. As already mentioned, all of these are 1 inch deep. It will be noticed that the sizes are minimal, and it may be necessary to add a few other sizes for convenience in mounting various kinds of specimens.

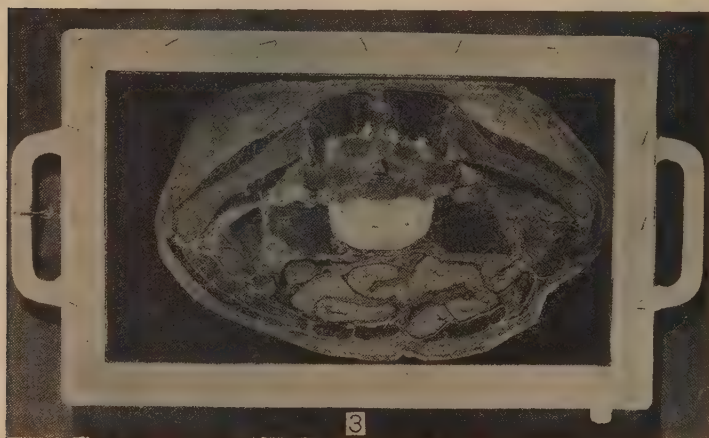


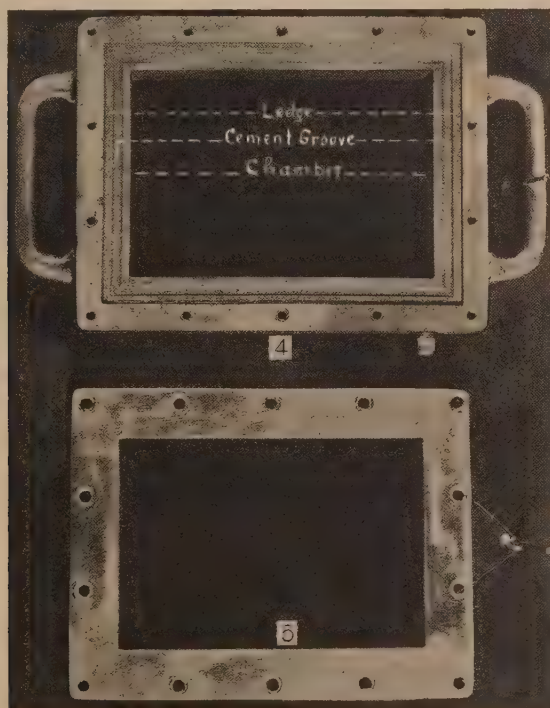
Figure 3

CONSTRUCTION OF CONTAINER FOR CROSS-SECTIONS OF THE BRAIN

The design of the container for sections of the brain is precisely the same as that for cross-sections of the body, except that the body is made thinner, so that there is only $\frac{1}{4}$ inch between glass plates. By this means thirty-two cross-sections of one brain can be mounted. The filling hole is of necessity smaller, only $\frac{3}{32}$ inch in diameter, which, however, is large enough to facilitate filling and refilling with relative ease. As to size, only one type has been thus far manufactured, which measures 6 inches wide and 8 inches long (fig. 2). It is satisfactory for cross-sections of the brain, but not for sagittal or horizontal sections, which should be 8 inches in width and 10 inches in length.

MOUNTING

The mounting process involves four principal phases, viz.: section cutting, sealing of containers, orientation of sections, and filling of containers.



Figures 4 and 5

Sections should be cut a little less than the depth of the chamber of the container. It has been found by experience that sections of the body should not exceed $\frac{7}{8}$ inch in thickness and those of the brain $\frac{3}{16}$ inch. In case the sections are the thickness of the ledge, air bubbles are eliminated with difficulty and the glass plate is liable to crack, especially if the section causes a slight pressure upon the glass. Care should be taken to see that sections are free from dust, debris, or any

kind of floating particles before they are mounted. It is best to submerge them for a few minutes in the same solution in which sections will be mounted permanently.

The first kind of sealing medium used for this container was a sort of aquarium putty, which consists of a mixture of—

Litharge,	1 pound
Whiting,	6 pounds
Red lead,	1 pound
Boiled linseed oil	

It is mixed to the consistency of ordinary putty, and can be easily handled in mounting. At present, however, it is no longer used for this purpose, as it has a few unfavorable features. With sudden fluctuation of temperature the formaldehyde becomes cloudy and evaporation is excessive. If the mounts are stored in ordinary room temperature, the objectionable features are minimal. A few of our mounts which have been put up with this putty are now fifteen months old and show a few air bubbles as well as a slight discoloration of the fluid.

After having tried a number of other sealing media, including Ozokerite, with similar or worse results, we are now using a cement made by mixing of—

Beeswax,	2 parts
Rosin,	1 part

The rosin is finely powdered and added gradually to the melted beeswax, so as to facilitate the melting of the former. The mixture may be prepared in larger quantities and melted whenever needed. Better results are obtained if the body of the frame is heated to a temperature slightly below the melting-point of the cement. The cement grooves on both sides of the frame are then filled and allowed to cool. It is convenient to do the filling with a wide-mouth pipette. Care must be taken to see that the groove is filled evenly and that the cement projects above the ledge approximately $\frac{1}{16}$ inch or slightly more. While the cement is cooling the glass plate is heated to a temperature a little above the melting-point of

the rosin. The glass plate is then placed in the depression of the frame and gently pressed down until the surface of the glass is even with or slightly below the surface of the frame. This can be best done by means of a straight-edge. If the mount is properly done, no air bubbles or streaks will be seen in the joint. The space between the glass and frame is next filled with cement, and, after it has cooled, the surplus is removed by means of a knife. The surface plate is then screwed on, and the container is ready to receive the section.

If proper orientation is desired, it can be accomplished in the following manner. Small holes can be drilled in the margin of the ledge to which threads supporting the material are tied. It is convenient to use a long and slender needle, by means of which the thread is pulled through the section in a crosswise manner and the ends tightened to the holes drilled in the frame (fig. 2). Small objects, like embryos, and many invertebrates may be suspended by a single thread.

After the material has been placed in the chamber of the frame and orientated, the second glass plate is heated and sealed in the same manner as its mate.

The filling is the last and the easiest part of the mounting process. After the cover-plates on both sides are securely tightened, the chamber is filled with 3 or 4 per cent formaldehyde. To remove the entrapped air bubbles the plug is screwed in temporarily and the container, held in both hands with the plug upward, is partially rotated until all the bubbles have left the material and gathered in the corner of the filling hole. More fluid is added until all air bubbles are expelled and the plug is screwed in permanently and sealed by a ring of cement.

Beeswax and rosin is by far the best cement we have used and the results are satisfactory. However, a cement which will seal these containers hermetically is still to be discovered. Small bubbles of air will seep in with time and a slight precipitate will form on exposure to fluctuations of temperature. If kept in ordinary room temperature, years may pass without enough bubbles or precipitate to become annoying. The old-

est mounts of this kind are ten months old and show a few air bubbles, but no precipitate. Before the mounts are handed to students, bubbles can be removed by opening the screw plug and adding a few drops of formaldehyde. Museum mounts will need no attention for several years.

OTHER APPLICATIONS

The container has been designed for cross-section anatomy material; this is only one of its various uses. There is hardly any museum specimen, unless too bulky, for which the container cannot be used with advantage. It is especially suitable for pathological, embryological, and parasitological material. The container is applicable for various kinds of comparative-anatomy sections and dissection, specimens of invertebrate as well as vertebrate zoölogy. Material can either be mounted in liquid or left dry, as is advantageous in various bone mounts and other material too fragile for safe handling.

MANUFACTURER AND PRICE

After extensive inquiry at the leading manufacturers of laboratory supplies of the United States, it was learned that the Kny-Scheerer Corporation of New York City offered the most reasonable terms. The containers were ordered in sets. A set for cross-sections of one body designated as series A and a set for the cross-sections of one brain, series B.

Series A 19 containers, 1" deep, 9" wide, 14" long
 2 containers, 1" deep, 9" wide, 18" long
 32 containers, 1" deep, 6" wide, 8" long

Series B 32 containers, $\frac{1}{4}$ " deep, 6" wide, 8" long

The price which the University of Oklahoma paid for one set, eighty-five containers, was \$750.00. The writer has corresponded with the company, and it said that the price may be somewhat reduced, depending upon the size of the order.

The price of these containers may, at first glance, appear high. If, however, the substantiality and neatness are considered and the fact that two levels are exposed in the one container, which is equivalent to two containers of other makes, the price is really very reasonable, indeed.

CORRELATIONS OF THE WEIGHTS AND LENGTHS OF THE BODY, SYSTEMS, AND ORGANS OF THE TURKEY HEN

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The average weights and lengths of the body, parts, systems, and organs of twelve bronze turkey hens, together with a statistical study of the variability of these measurements, have been published in an earlier number of this journal. These data give no information concerning the relationship of the weight and length of the individual parts, and so this little report has been prepared to give some idea of the correlated variability of these organs and systems. Of course, with this very limited number of specimens no final conclusions may be drawn.

The history of the specimens and the method of autopsy have been given in the earlier report (Latimer and Rosenbaum, '26) and will not be repeated here. The formulae used are those given by Davenport ('14). The weights of the various systems and organs will be correlated with the net body weight, then the weights of some of the systems will be correlated with their parts, and, next, correlations between the weights of various organs. Then the correlations between the lengths of the body and of various parts will be given, and, lastly, a summary of various anthropometric indices will be shown.

CORRELATIONS WITH BODY WEIGHT

The correlations with the net body weight are given in table 1. The net body weight, or the gross weight minus the weight of the contents of the digestive tube, was used in

making these correlations, for this is the actual weight of the substance of the turkey and does not include a variable amount of material in the digestive tract, which is really not a part of the turkey. This table shows the eyeballs with the highest correlation, and the heart and integument next in order. The second group of three, namely, musculature, digestive tract, and ligamentous skeleton, form large percentages of the body weight, and so might be expected to have high correlations, which the table confirms. In this first report on the turkey it was shown that the musculature forms an average of 59 per cent of the entire weight of the

TABLE 1
Correlations with the net body weight. All weights are in grams

Weight, eyeballs	+0.87±0.05	Weight, brain	+0.61±0.12
Weight, heart	+0.85±0.05	Weight, spleen	+0.48±0.15
Weight, integument	+0.85±0.06	Weight, oviduct	+0.43±0.16
Weight, musculature	+0.83±0.06	Weight, ovary	+0.40±0.16
Weight, empty digestive tract	+0.81±0.07	Weight, pancreas	+0.25±0.18
Weight, ligamentous skeleton	+0.80±0.07	Weight, lungs and trachea	+0.20±0.19
Weight, kidneys	+0.79±0.07	Weight, hypophysis	—0.07±0.19
Weight, liver	+0.75±0.09	Weight, feathers	—0.21±0.19
Weight, spinal cord	+0.73±0.09	Weight, thyroid	—0.56±0.13
Weight, head	+0.71±0.10	Weight, thymus	—0.60±0.12
Weight, suprarenals	+0.69±0.10		

turkey; the ligamentous skeleton, 10 per cent, and the empty digestive tract, 5 per cent. The first six entries in this table, all of which have a positive correlation of more than 0.8, together form 83 per cent of the total weight of the turkey. The two which have the highest correlation, the eyeballs and the heart, form but 0.24 and 0.53 per cent, respectively.

It is interesting to note that the correlation between the spinal cord and the body weight is one and a half times the correlation of the brain and the body weight. It has been shown that the growth of the spinal cord follows the general body growth in the chicken more closely than the growth of the brain (Latimer, '25). The correlations of the brain and the cord with the body weight for the turtle and the dog (Latimer, '24 b) show the same relations.

The two lowest positive correlations in this table are for the pancreas and the combined weight of the lungs and trachea. The correlation of 0.25 for the pancreas is lower than the correlation of 0.59 given by Cameron ('26) for the normal guinea-pig, or 0.39 for the turtle and the high correlation of 0.91 for the frog (Latimer, '20). The low correlation between the lungs and the body weight may be accounted for, in part at least, by the great variability in the weight of the lungs, due probably to the varying amount of blood contained in the autopsied lungs. The similar correlation for the turtle lungs is lower than this correlation for the turkey lungs.

The last four correlations in this table are negative, and the first two of these, or the correlations for the hypophysis and the feathers, are so low that we may say that there is no correlation between the weight of the body and the weight of the hypophysis or of the feathers. There was an error in table 1 of the first report (Latimer and Rosenbaum, '26) in the hypophysis. The decimal point should be one place to the right of its present position in the probable error of the average, in the standard deviation and its probable error, and in the coefficient of variability and its error. The correlations for the thyroid and the thymus are low, but still valid negative correlations. The growth curves for the feathers and the thymus of the chicken (Latimer, '24 a) both show a decrease following puberty, while the body weight is still increasing, and this is what is happening in these turkeys which were autopsied at the beginning of their first laying period.

Table 2, A, gives the correlations of the entire digestive tube with its parts. The correlations between the digestive tract and the liver and the pancreas are also included in this table. The gizzard and intestines form, respectively, 52 and 31 per cent of the weight of the entire digestive tract, and hence we may expect a high correlation between these parts and the digestive tract. An error was made in the standard deviation and the coefficient of variability of the gizzard.

These values should be 8.95 ± 1.23 for the standard deviation and 9.32 ± 1.30 for the coefficient of variability, in place of the values given in table 1 of the first report (Latimer and Rosenbaum, '26). The correlation of the weight of the empty digestive tract and the glandular stomach is the lowest in table 2, A, with the esophagus (including the crop) next.

Greenwood and Brown ('13) have studied the correlations between the weights of the entire body, and the heart, kidney, liver, and spleen. In table 3 of their report the relative order of correlations with the body weight is first or highest, heart, then liver, kidney, and brain. It will be seen from table 1 that this order holds for the turkey hen, with the exception of the kidney and liver, which are reversed in order in the correlations for the turkey hen. The correlations of the kidney, the liver, and the spleen with the heart weight are in this order with the first the highest correlation as given for man in the above-mentioned paper. Table 2, B, shows the correlations of the heart with the kidney highest, followed by the correlation with the liver and the spleen, which is the same order as that for man. The correlations with the digestive tube (without contents) and the lungs have been added. The correlation with the heart and lungs is the lowest, due possibly to the variable amount of coagulated blood found after death in the lungs. The correlations of the heart and the kidney and liver indicate a rather close relationship between the heart and each of these organs.

Table 3 gives the correlation of the ovary and the oviduct in the first line, and, as might be expected, it is a very high positive correlation. Following this is the correlation of the thymus and feathers—a low positive correlation. The next two are negative, and the last one, namely, that between the thymus and the ovary, is a significant negative correlation, showing that as the ovary increases in size just before the beginning of the first laying period the thymus is actually decreasing in size. It has been shown (Latimer, '24a) that this statement holds in the growth of the thymus and the ovary of the chicken.

Table 4 shows the weights of five of the long bones correlated with the weight of the entire skeleton in the second column and with the total body length in the last column. All of the weights of the skeletal elements are in grams and

TABLE 2

A. Correlations of the weights of the empty digestive tube and its parts

Esophagus.....	+0.49±0.15
Stomach (glandular)	+0.48±0.15
Gizzard.....	+0.83±0.06
Intestines.....	+0.80±0.07
Liver.....	+0.53±0.14
Pancreas.....	+0.52±0.14

B. Correlation with the heart weight

Weight, kidney.....	+0.72±0.09
Weight, liver.....	+0.72±0.10
Weight, spleen.....	+0.56±0.14
Weight, digestive tract	+0.54±0.14
Weight, lungs.....	+0.44±0.16

TABLE 3

Correlations of organ weights

Ovary and oviduct	+0.97±0.01
Thymus and feathers	+0.37±0.17
Thymus and hypophysis	-0.47±0.15
Thymus and ovary	-0.61±0.12

TABLE 4

Weights in grams of the long bones correlated with skeletal weight in grams, and body length measured in millimeters

BONE WEIGHT, GRAMS	SKELETAL WEIGHT	BODY LENGTH
Humerus.....	+0.76±0.08	+0.50±0.15
Radius.....	+0.97±0.01	+0.78±0.08
Femur.....	+0.92±0.03	+0.91±0.03
Tibia and fibula.....	+0.92±0.03	+0.93±0.03
Tarsometatarsus.....	+0.88±0.05	+0.75±0.09

the lengths in millimeters, and both taken in the ligamentous condition. These are all high positive correlations. In a study of the growth of the skeleton of the chicken (Latimer, '27) it has been shown that, when the weights of the bones are reduced to percentages of the skeletal weight, these per-

centages are fairly constant. Likewise, the weights of the radius and ulna plotted on gross body weight form straight lines, thus showing a constant relationship to the weight of the entire body, and the weights of these two bones reduced to percentages of the entire body remain constant throughout the latter part of the growth period. In both the turkey and the chicken the weight of the radius, especially, is a good criterion of the weight of the skeleton and of the entire body. In both the chicken and the turkey hen the weight of the bones of the leg are more closely related with the weight of the entire skeleton than are the bones of the wing.

The coefficient of variability of the tarsometatarsus of the turkey hen was given incorrectly in the first report (Latimer and Rosenbaum, '26), and this should read 9.13 ± 1.31 . This means that the humerus and not the tarsometatarsus is the least variable in weight of the six long bones.

The correlations between these weights and the total body length, given in the last column of this table, are lower, with one exception, than the correlations with the skeletal weight, or we may say that the weights of these bones are better indicators of the weight of the skeleton than of the body length, and that the bones of the leg form higher correlations, both with the skeletal weight and with the body length, than do the bones of the wing. The lowest correlation in both columns is that with the humerus, or the weight of the humerus is the poorest indicator of both the skeletal weight and body length.

CORRELATIONS BETWEEN THE LENGTHS OF BODY AND ORGANS

Table 5 gives the correlations between the body length measured in millimeters and the lengths of the digestive tract and its parts also measured in millimeters in the second column, and the third column gives the correlations between the length of the digestive tract and its parts. It is interesting to note that the correlation between the length of the digestive tract and the body length is but 0.45, while the correlation between the weight of the digestive tract and the

body weight, as given in table 1, is 0.81, or 1.8 times greater. The correlation between the weight of the digestive tube and the weight of the esophagus, given in table 2, A, is much higher than the correlation for the lengths of the similar parts, but the length of the intestines has a higher correlation with the length of the digestive tract than the weights of intestine and digestive tract. The high correlation between the lengths of the digestive tube and the intestines may be due in large part to the fact that the intestines form 85 per cent of the total length of the digestive tube. The coefficient of variability for the total length of the digestive tube was not given in the other paper, and it is interesting to see that it averages 2231.5 mm. in length and its coefficient

TABLE 5

Lengths of the parts of the digestive tract correlated with body length and total length of the digestive tract

LENGTH	BODY LENGTH	DIGESTIVE-TRACT LENGTH
Digestive tract.....	+0.45±0.16	+1—
Esophagus.....	+0.31±0.18	+0.14±0.19
Intestines.....	+0.36±0.17	+0.96±0.02
Caeca.....	+0.50±0.15	+0.55±0.14

of variability is but 5.56 ± 0.77 or less than that for the esophagus and for the intestines. The length of the esophagus has the highest coefficient of variability of any of the parts of the digestive system, and the lowest correlation with the body length and the length of the entire digestive tract; and its weight (including the weight of the empty crop) has the lowest correlation with the weight of the entire digestive tube (table 2, A), with the exception of the glandular stomach, which is very slightly lower.

The second column of table 6 gives the correlations of the body length with the lengths of the long bones, the length of the left leg, and the left wing, and the length and width of the head. In the third column are given the correlations of the head length with the same lengths, and in the last column, the similar correlations with the weight of the skeleton.

The lengths of the three wing bones give the highest correlations with the skeletal weight, next with the body length, and the lowest with the head length. The lengths of the three bones of the leg have the highest correlations with the body length, next with the head length, and the lowest, with the skeletal weight, except for the tarsometatarsus for which the lowest correlation is with the head length and the skeletal weight second. The wing length is most closely correlated with the skeletal weight, then body length, and last head length, thus following the individual bones of the wing. The total length of the leg has the highest correlation with the body length. The average of the correlations with the head length is lower than the average for the other two columns. The head width, in next to the last line, is poorly correlated with body length and skeletal weight, and the correlation with the head length, is but little higher.

Schneider and Dunn ('24) have studied the bones of a much larger number of Leghorn chickens, and in general their correlations agree with the results found in this report, except that their correlation between the humerus and femur is much higher than that found in the turkey, and all of their correlations between individual bones are higher. They have correlated the lengths of the humerus and femur with the length of the skull after the bones and the skull had been cleaned, while in this paper the lengths of the cleaned bones are correlated with the head length, which was taken immediately after the turkey was killed. They give a correlation of 0.61 between the lengths of the cranium and the humerus and 0.57 between the cranium and femur.

Orensteen ('15) gives a correlation of 0.24 between the head length and breadth of 802 Cairo-born men, and a list of similar correlations, ranging from 0.08 to 0.47 for seven other races, which brings this correlation of 0.37 for the turkey slightly above the average of these correlations for the various races of men. MacDowell ('14) gives four correlations with skull length of the rabbit, namely, skull length and the length of the humerus, femur and tibia, and all of these correlations

are higher than the similar correlations for the turkey. Castle ('22) finds the highest correlation in the rabbit between the length of the long bones.

Table 6 shows that the correlations between the lengths of the wing bones and the skeletal weight are much higher than the similar correlations for the leg bones, but when the weights of these bones are correlated with the skeletal weight and the body length, as given in table 4, the average correlation for the leg bones is greater, although the highest cor-

TABLE 6

Lengths of the long bones and parts correlated with body length, head length, and skeletal weight

bone length, mm.	body length, mm.	head length, mm.	skeletal weight, grams
Humerus	+0.77±0.08	+0.55±0.14	+0.85±0.05
Radius	+0.65±0.11	+0.46±0.15	+0.82±0.06
Ulna	+0.76±0.08	+0.64±0.12	+0.83±0.06
Femur	+0.66±0.11	+0.65±0.11	+0.39±0.17
Tibia	+0.83±0.06	+0.68±0.11	+0.57±0.13
Tarsometatarsus	+0.89±0.04	+0.71±0.10	+0.76±0.08
Carina	+0.82±0.06	+0.65±0.11	+0.62±0.12
Wing	+0.62±0.12	+0.59±0.13	+0.70±0.10
Leg	+0.91±0.03	+0.75±0.09	+0.78±0.08
Head	+0.70±0.10	+1—	+0.59±0.13
Head (width)	+0.27±0.19	+0.37±0.17	+0.22±0.19
Body length	+1—	+0.70±0.10	+0.80±0.07

relation is for the weight of the radius and the skeletal weight. The weights of the individual bones are more closely correlated with the skeletal weight than the body length, although for the leg bones the difference is not so great as for the two wing bones. A comparison of the two tables also shows that the weights of the long bones are more closely correlated with the skeletal weight than the lengths of these bones.

Table 7 shows the correlations between the lengths of the wing and the leg and the correlations between the various bones of these appendages. The highest correlation (0.93) is between the two wing bones; the radius and ulna, the

second highest, between the humerus and the ulna, and the lowest, which is but 0.20, between the humerus and the femur. The correlations for three pairs of bones given by Schneider and Dunn ('24) are all higher than the similar correlations for the turkey, but they follow the same order. They are as follows: humerus and ulna, 0.94; femur and tibia, 0.93, and the humerus and femur, 0.90. This last correlation, it will be noticed, is much higher than the similar correlation for the turkey, although it is the lowest of the three correlations for the individual chicken bones. Castle ('22) gives the correlation of 0.91 between the lengths of the humerus and femur. In both the turkey and the chicken the highest correlations are between bones in the same extremity, and the correlations of the wing bones are higher than those of the leg, and the lowest correlation is between the homologous bones of the two extremities.

ANTHROPOMETRIC INDICES

Table 8 gives the average, the range, the standard deviation, and the coefficient of variability for eight anthropometric indices. The first of these is the intermembral index or the ratio of the sum of the lengths of the humerus plus the radius divided by the sum of the lengths of the femur plus the tibia. The next two indices, or the humerofemoral and the radio-tibial, compare the lengths of the bones in the homologous divisions of the wing and lower extremity, and the fourth and fifth ratios compare the individual bones in the wing, and the last three ratios are those of bones in the lower extremity. The coefficients of variability, given in the last column, show that the humerofemoral index varies the most, and the radio-ulnar index is the least variable.

Comparison of these indices with similar indices of the Leghorn chicken (Latimer, '27) shows some interesting variations. The first three indices in this table are from 6 to 15 per cent greater in the turkey than in the chicken, showing that the wing, the humerus, and the radius are relatively longer in the turkey than in the chicken. The greatest difference

is in the humerofemoral index, which is but 90 per cent in the chicken and 105 per cent in the turkey. The smallest difference is in the radiotibial index, which is 56 per cent in the chicken and 62 per cent in the turkey. When the lengths of the bones in the same extremity are compared, the ratios for the turkey are about the same as those for the chicken, the

TABLE 7

*Correlations of the lengths of the leg and the wing
and of the individual bones*

Leg and wing	$+0.81 \pm 0.07$
Humerus and femur	$+0.20 \pm 0.19$
Humerus and ulna	$+0.87 \pm 0.05$
Radius and ulna	$+0.93 \pm 0.03$
Femur and tibia	$+0.53 \pm 0.14$

TABLE 8

*The average, the range, the standard deviation, and the coefficient of variability
of the anthropometric indices*

	AVERAGE	RANGE	STANDARD DEVIATION	COEFFICIENT VARIABILITY
Intermembral	0.789 ± 0.005	0.74—0.83	0.025 ± 0.003	3.13 ± 0.43
Humerofemoral	1.05 ± 0.01	0.92—1.10	0.045 ± 0.006	4.29 ± 0.59
Radiotibial	0.615 ± 0.004	0.58—0.66	0.023 ± 0.003	3.70 ± 0.51
Radiohumeral	0.889 ± 0.004	0.86—0.94	0.020 ± 0.003	2.27 ± 0.31
Radio-ulnar	0.897 ± 0.002	0.88—0.92	0.011 ± 0.002	1.20 ± 0.17
Tibiofemoral	1.53 ± 0.01	1.42—1.61	0.051 ± 0.007	3.33 ± 0.46
Femerotarsometatarsal	0.982 ± 0.006	0.94—1.07	0.031 ± 0.004	3.17 ± 0.44
Tarsometatarsotibial	0.668 ± 0.003	0.65—0.69	0.013 ± 0.002	1.98 ± 0.27

differences being less than 5 per cent in every case. This seems to indicate that the wing and the two bones of the wing are relatively longer in the turkey than in the chicken when their lengths are compared with the lengths of the lower extremity and the femur and tibia, respectively, and that the relative proportions of the wing and the leg are the same in both the chicken and the turkey.

SUMMARY

The correlations between the net body weight and the weights of the organs range from a high positive correlation of 0.87 for the eyeballs to a negative correlation of 0.60 for the thymus. The six parts with the highest correlations form 83 per cent of the total weight of the turkey. The heart forms but 0.5 per cent of the total weight, yet it has the second highest correlation with the body weight.

The correlations of the heart with the kidney, liver, spleen, and digestive tract are all good positive correlations.

The weights of the ovary and oviduct have a very high positive correlation, and the correlation between the ovary and the thymus is a significant negative correlation.

The weights of the three leg bones form higher correlations with both the skeletal weight and body length than do the wing bones. The length of the esophagus has the lowest correlation of any part of the digestive tract with both the body length and the length of the entire digestive tract, and its weight and the weight of the entire digestive tract is next to the lowest correlation of any of the parts of the tract.

The skeletal weight is more closely correlated with the lengths of the wing bones and the weights of the leg bones, and for all of the long bones the correlations between the skeletal weight and bone weight are higher than between the skeletal weight and bone length.

The higher correlations in the lengths of the individual bones are between bones in the same extremity.

The anthropometric indices show that the wing and the individual bones of the wing are relatively longer in the turkey than in the chicken.

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OBSERVATIONS ON GERM-CELL ORIGIN IN THE ADULT MALE TELEOST, UMBRA LIMI

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TWO PLATES (TWELVE FIGURES)

In a recent paper (Foley, '26) it was pointed out that in the teleost, *Umbra*, spermatogonia are formed anew each season from cells which migrate from some undetermined position outside the bounds of the testis lobule. The purpose of the present inquiry was to ascertain, if possible, the source and mode of origin of these migrating cells.

The material upon which the present observations are based, consisting of sexually mature individuals of the mud-minnow (*Umbra limi*), was collected in the vicinity of Madison, Wisconsin. Collections of the living fish were made at least once a week, and material was fixed daily or on alternate days from the 1st of May until the middle of September (in 1923 and 1924). As pointed out later in the discussion, this period embraces all the essential phenomena in the seasonal cycle of the germ cells. Some of the fish were killed, split open along the midventral line, pinned down to a wax pan, and entirely covered with the fixative. If this method be not followed, the testis curls when immersed in the fixative, rendering sectioning and future interpretation of sections difficult. Other testes were not fixed entire, but were quickly immersed in the fixative and cut into very small pieces. Although the latter method gives the better cytological differentiation, satisfactory fixation is secured by fixing the entire testis. Sections were cut from 3μ to 10μ in thickness. Sagittal sections of the entire testis, 10μ in thickness, proved most valuable in this study.

Allen's modification of Bouin's fluid was found to give the best fixation, the fixative being kept at the same temperature as the water of the aquarium which contained the fish. Although other nuclear and cytoplasmic stains were employed, the best results were obtained with iron-alum hematoxylin counterstained with lichtgrün or Congo red. Mallory's triple stain was employed to differentiate the mesothelial covering of the testis from the underlying connective tissue.

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ANATOMY OF THE TESTIS IN UMBRA

The testes are two elongated sac-like bodies situated in the posterior part of the body cavity just ventral to the swim bladder. They are oval in shape, tapering from the middle toward each end. In Umbra the testes do not fuse as they do in many fish, but remain separate throughout their entire length. Each testis is attached to the swim bladder by a delicate mesorchium. The testes show no relation to the wolffian bodies; posteriorly they communicate independently with the cloaca by separate vasa deferentia. The vasa deferentia enter the cloaca just lateral to a ventral bladder-like evagination of the intestine.

The entire testis is covered by peritoneum, the mesothelium of which (figs. 1, 3, and 4 to 12) is a distinct though very delicate membrane resting upon the underlying fibrous tissue. The mesothelium remains distinctly a thin simple squamous epithelium throughout the entire cycle of spermatogenesis, never being elevated into a cuboidal or columnar layer. Analysis of the peritoneal components can be made by differential staining with Mallory's triple stain. After this stain, nuclei and cytoplasm are red or yellow and the fibrous tissue a dark blue. The mesothelial nuclei are of a vesicular character, containing a variable number of karyo-

somes scattered along the linin network. The nuclei in the sections are spaced at variable intervals in the membrane, ranging from $20\ \mu$ apart, when the testes are relatively small in volume, to as high as $2000\ \mu$ apart at the completion of the spermatogenetic cycle when the testes are at their greatest distention. The cytoplasm of the mesothelial cells between adjacent nuclei is extremely thin, extending as a mere homogeneous film over the surface of the testis.

The fibrous stroma of the testis is of a very diffuse nature. It is beyond the scope of this paper to enter into a discussion of the varieties of fibrous tissues making up the stroma; however, the capsule, lobular and intralobular septa are predominantly of the white fibrous variety. The capsule, although loose in texture at all times, presents its most diffuse arrangement of fibers during the height of migration of the new crop of germ cells into the lobules. At this time the capsule has an average thickness of about $120\ \mu$. Later, as the testis increases in volume, the capsule of the testis becomes more compact and gradually is reduced in thickness until at the end of the spermatogenetic cycle an almost inappreciable stratum of fibrous tissue intervenes between the mesothelium and the germ cells within the engorged lobules subjacent to the capsule. The loose texture or extreme thinness of the capsule is of direct importance if it is to be considered a pathway by which germ cells might migrate from the mesothelium into the interior of the testis.

Trabeculae of connective tissue extend inward from the capsule and divide the testis into lobules. The lobules are in turn subdivided into cysts by secondary septa. These secondary septa are best observed during the multiplication period, which is at its maximum from the middle of July through the first week in August. Previous to this time, the fibrous tissue surrounding the early spermatogonia, each of which is the center of a future cyst within the lobule, is quite diffuse (figs. 4, 5, and 6), but, with the proliferation of each early spermatogonium to form its cyst, this loose fibrous tissue becomes compacted into an intercyst septum or cyst wall.

If the lobules be examined from the onset of the period of spermiogenesis, which begins during the first week in August, through the final phase of spermatozoon formation, it will be seen that there are no cells other than spermatozoa present in cysts which have just completed spermiogenesis (fig. 1). In a former paper it was stated that the spermatogonia, which form each seasonal crop of spermatozoa, take origin from cells which migrate into the lobules. This earlier statement applied only to the origin of germ cells within old lobules. Although cyst formation, in the main, occurs in old lobules from which the spermatozoa have been expelled, subsequent observations have shown that cysts may be formed in the connective tissue outside persisting lobules (figs. 5 and 6). In figure 5 may be seen three lobules, of which two show definite boundary lines; the other exhibits indistinct separation from the capsular connective tissue and can be assumed to have been formed by cells undergoing transformation in the stroma between two old lobules. Figure 6 shows the beginnings of a new lobule in the capsular connective tissue. Whether cysts are formed within new or old lobules, the principle is the same; a single cell enlarges as an early spermatogonium and later undergoes mitosis to form the content of the cyst. In the main, if the transformation is taking place within an established lobule, each early spermatogonium is formed by growth enlargement of a cell which has entered the lobule. However, transformation does take place in the fibrous tissue outside persisting lobules by the growth of a stroma cell into a spermatogonium. These early spermatogonia are from three to six times the size of the final spermatogonia which transform into spermatocytes. Thus the spermatogonia may be grouped into two classes: the initial, large ones are designated as primary spermatogonia, while the much smaller ones, which are formed by multiplication of a single primary cell, are called secondary spermatogonia. Once established, each of these primary spermatogonia eventually becomes the center of a cyst within the lobule. Most commonly, the products of the division of each of these

early or primary spermatogonia will form one cyst, but frequently the product of the earlier divisions of these primary spermatogonia may each grow into a primary one and undergo mitosis forming the content of separate cysts.

This process of cyst or pocket formation begins at the periphery and gradually extends toward the center of the lobule until the latter is filled with a solid cord of germ cells. Further details of cyst formation are concerned solely with the multiplication of these primary spermatogonia and their descendants to form the small spermatogonia which transform into primary spermatocytes at the beginning of the maturation divisions. At the completion of spermiogenesis within the cysts of the lobule, the cyst walls disappear and are not reformed until the beginning of the next seasonal cycle. If the lobules of figure 5 be examined, it will be seen that the space between the primary spermatogonia is filled with fibrous connective tissue which later forms the walls between the cysts.

Since the cyst walls disappear after the lobules become filled with spermatozoa (center of fig. 4) and are not reformed until the next season, the question of the origin of this intra-lobular fibrous tissue presents itself for consideration. The connective tissue which occupies persisting lobules results in part from the action of migrating germ cells which push fibers of the interlobular trabeculae into the lobule (fig. 4). However, considering the amount of fibrous tissue present after the reorganization of the lobules, which is accomplished through the formation of cysts, it does not seem plausible to conclude that all the fibrous tissue arose in this manner. In all probability a considerable portion was produced by cells acting as fibroblasts within the lobule. When new lobules are formed by nests of germ cells transforming in the stroma, the lobular and cyst walls will be formed mainly by a compacting of the fibrous tissue already present (fig. 6, and indefinitely bounded lobule of fig. 5), and, as in the case of established lobules, there is probably an additional intra-lobular augmentation of fibers.

THE PRESENT STATUS OF GERM-CELL ORIGIN IN VERTEBRATES

Interpretations of germ-cell origin in the adult organism are naturally interrelated with the earlier embryonic history of the germ cells. It is beyond the scope of this paper to enter upon a discussion of the opposed views which have been expressed with respect to the early germ-cell history in vertebrates. Hargitt ('24) reviews this aspect of the question, and for the present purpose it is sufficient to state the two contrary interpretations. On the one hand, it is asserted that the primitive germ cells are segregated at the very beginning of embryonic development, later migrating by way of a definite germ tract into the gonad. Others hold that germ cells arise in later ontogeny through processes of differentiation from mesenchymal or peritoneal elements. Applied to the adult, and under conditions of the reproductive cycle obtaining in *Umbra*, the question at issue is as to how the new crop of germ cells is formed at the beginning of each seasonal cycle: whether they are produced from reserve germ cells residual in the lobule, which in turn are descended from other reserve cells, or whether they are formed anew from migrating cells which come from some point outside the lobule.

Only incidental reference has been made to the origin of the germ cells of adult teleosts. Turner ('19), working on the adult perch, asserts that the annual crop of spermatogonia comes from cells which enter the depleted lobules, as I have determined in *Umbra*, but in neither species has the origin of these cells been traced. As a subject of investigation, germ-cell origin in the adult vertebrate has been dealt with to a greater extent in the amphibians than in any other class of vertebrates. Hargitt ('24) gives a review of the work done on this group. In his own study of germ-cell history in the adult *Diemyctylus*, Hargitt records in this form a seasonal replacement of spermatogonia from sources outside the cyst—a finding in general similar to the present finding in *Umbra*, although differing in the sites from which the replacing cells arise. In *Diemyctylus* the migrating cells in

the vicinity of the collecting ducts are derived from the epithelium of these ducts, and in regions remote from the collecting ducts the germ cells originate from the peritoneal epithelium covering the testis. In addition, he says, "the germinal epithelium and its neighboring stroma seem to be equally plastic and cells of either or both may undergo modifications which result in the production of spermatogonia." Considering the similarity in structure of the testis of amphibians and teleosts, a source of origin either from the collecting tubules or the epithelium covering the testis might be inferred in Umbra. Since the testis of Umbra does not contain a system of ducts comparable to the vasa efferentia or collecting ducts, the scope of the problem is limited to a consideration of germ-cell origin from sources other than the wall of the ducts.

RESERVE GERM CELLS

It should be pointed out that the term cell is often used or implied here and elsewhere in the discussion when referring to the stroma cells and very early spermatogonia within the lobules, whereas in most cases only the nuclei of these cells are shown in the figures. The limits of the cytoplasm in such cases are obscured by the fibrous tissue which surrounds the cells.

In a discussion of the seasonal cycle in Umbra (*loc.cit.*) it was pointed out that expulsion of the spermatozoa occurs during the last week or ten days in May. At this time the migration of cells is at its height. A migrant, as here considered, is a cell which resembles a spermatogonium within the lobule and, from its position immediately bordering the lobule, suggests the possibility of its entrance. In their size, form, and staining reaction of their nuclei, the migrants resemble the early stages of the transforming spermatogonia within the lobule (fig. 4, *E* and *D*). In the original description of these migrant cells (*loc.cit.*) it was stated that they are oblong or flattened, with a prominent chromatin nucleolus suspended in the reticulum near the nuclear membrane and with

the chromatin granules, which are quite large, arranged peripherally against the nuclear membrane. This earlier account pertained only to those cells which had attained a position within the lobules, and hence were not in the strict sense of the word migrants, but rather actual spermatogonia. Subsequent observations have shown that the aforementioned description is not even typical for the early stages in spermatogonial formation. As shown in the text figures, the nuclei of both migrants (fig. 4, *E* and *D*) and early stages of transforming spermatogonia (fig. 4) have their chromatin distributed along the linin network. With only occasional exceptions (figs. 2 and 5, *F*), the chromatin of the nuclei of spermatogonia is not collected into a definite chromatin nucleolus until the cells have undergone considerable growth. The majority of the testicular lobules of mature individuals of *Umbra* have completed their cycle of spermatogenesis by the middle of September. During the interval from the last of September until the last of May the testis is engorged with spermatozoa. Expulsion of the spermatozoa usually occurs during the last week or ten days of May. Preceding and just following expulsion of the spermatozoa, cells varying in their nuclear organization from the type of migrants to growing and large resting spermatogonia are arranged around the inside of the lobule (fig. 4). It might be inferred that these cells constitute a germinal residuum, that they are the product of reserve germ cells which have existed in the lobule as such since the formation of the first or primordial germ cell in early embryogenesis. However, contraindicative evidence is obtained if the lobules be examined from the onset of the period of spermiogenesis, which begins during the first week in August, through the final phases of spermatozoon formation. It will be seen that in the cysts which have just completed spermiogenesis (fig. 1) there are no cells whatever present from which cells such as shown in figure 4 might have been derived. Such appear later, after the process of spermiogenesis has been completed in all or the majority of the cysts of any one lobule (fig. 2).

If these cells do not originate from reserve cells, the question arises as to just what is their origin. Cells considered to be of the migrant variety (fig. 3, *H*), together with transforming or resting spermatogonia (fig. 3), may be found in the connective tissue of the interlobular trabeculae near the periphery of the lobules which do not contain such enlarged cells. Cell *H* of figure 3 appears to be initiating migration into the lobule. Presumably cells similar to this one gave origin to the four spermatogonia of figure 2. The large resting spermatogonium of figure 3 apparently originated from a cell similar to *H* which, instead of migrating into the lobule, underwent spermatogonial differentiation in the fibrous tissue of the capsule. The nuclei of resting spermatogonia, as noted above, are of a distinctly vesicular nature containing a prominent chromatin nucleolus (figs. 3 and 5). Figure 4 shows a lobule just before the expulsion of the spermatozoa; the cells lining the inner border of the lobule, in various stages of development, range from the migrant type (*E* and *D*) to almost fully formed spermatogonia. Cells similar in size, shape, and staining reaction to those within the lobule may be seen in the interlobular trabeculae. Cells *E* and *D* in the figure appear to be actively engaged in migrating into the lobule; their position in the interlobular trabeculae and their resemblance in size, form, and staining reaction to spermatogonia within the lobule point to them as migrants. The evidence presented appears to justify the conclusion that cells similar to *D* and *E* gave origin to the spermatogonia within the lobule of figure 4. It should be further noted that other stroma cells of this figure likewise present the possibility of adding to the spermatogonia within either this or more remote lobules, judging from their resemblance in size, shape, and staining reaction either to the migrants or transforming spermatogonia within the lobule. The absence of cells within cysts after the completion of spermatogenesis, together with the observed transformation of migrant cells, points to the absence of a definitive germinal stock as the source of the seasonal crop of germ cells.

Since the spermatogonia do not take origin from definitive reserve cells, it might be argued that other cellular elements of the testis, such as epithelial cells of the collecting ducts, nurse cells, the cells of the testicular mesothelium, or stroma cells serve as a source for the annual crop of germ cells. The first two possibilities may be dispensed with in this investigation as neither collecting ducts nor nurse cells are present in *Umbra*.

TESTICULAR EPITHELIUM

All the figures are drawn to the same magnification; therefore, size differences between mesothelial nuclei at different periods of activity can be seen by reference to the figures. The character of the mesothelium covering the testis is subject to considerable variation. Usually, the mesothelial nuclei are elongated, oval, and irregular in outline when observed in sections vertical to the mesothelial surface (figs. 4 to 8). As a rule, such nuclei are more chromophilic than the ones which are to be described as possible migrating mesothelial nuclei. Marked changes occur in the mesothelial tissue covering the testis once the spermatozoa are expelled from the lobules. While these activities may be correlated with changes in size and contour of the testis due to the expulsion of the spermatozoa and subsequent relaxation of the tension heretofore exerted on the mesothelium, on the other hand some of the changes may possibly indicate changes toward an actual migration of cells from the mesothelium.

Following expulsion of the spermatozoa, the nuclei increase in size (figs. 10 to 12) and become more lobed and irregular of outline than nuclei of the resting mesothelium (figs. 4 to 8). Such changes in size and form might be attributed to the decrease in volume of the testis. The resultant lessening of the tension on the mesothelium would provide for the apparent increase in size of the mesothelial nuclei, while the inward pull of the fibrous tissue on the mesothelium together with the inherent contractility of the cells themselves could produce the lobulated form of the

nuclei (figs. 10 to 12). However, similar appearances as to shape may be observed in mesothelial nuclei (figs. 8 and 9) covering areas of testis which contain growing spermatogonia and where it is certain that the changes in form of the nuclei do not indicate migration. Here the mesothelium is more taut than that covering areas of testis which are devoid of distending elements. Figures 7 to 9, all taken from different regions of the same testis and all covering distended lobules, show mesothelial nuclei of various shapes. The mesothelial nucleus shown in figure 7 is elongated and presents a fairly regular outline. The mesothelium at this point was tightly stretched over the surface of the testis. Figure 8 shows a spermatogonium undergoing transformation in the stroma just beneath the mesothelium. It will be observed that the mesothelial nuclei on either side of the spermatogonium show slight extensions into the stroma. This effect is probably produced through the pull exerted on these cells by the fibrous tissue and indirectly caused by the pushing outward of the mesothelium by the growing spermatogonium. Figure 9 shows mesothelial nuclei of pronounced irregularity, one having assumed a crescentic or horseshoe shape which gives the appearance of initiating migration, although it is still in connection with the mesothelium; the effect produced being due to the contraction of the mesothelium at this point. The nucleus immediately adjacent to the one described shows a similar shape, although with less degree of contortion. Figure 9 was drawn from a region of testis near the point of attachment of the testis to the swim bladder. Contraction of the mesothelium of the testis is always pronounced at this point, while regions of testis more remote do not show pronounced irregularity. Although the nuclei of figures 8 and 9 show resemblances in form to some of the nuclei of figures 10 to 12, it is certain that the changes of form in the nuclei of these figures are not indicative of migration, but are due either to traction (fig. 8) or release of traction (fig. 9) on the mesothelium or the underlying stroma.

Coincident with the increase in size and change in shape of their nuclei (figs. 10 to 12), there is an occasional appearance suggesting that some mesothelial cells may migrate from the epithelium covering the testis into the stroma. If such appearances indicate migration at all, it is clear that cell division does not approximately precede the time of migration. Mitotic figures are of common occurrence in the spermatogonia of the fibrous stroma of the capsule, but all the material examined, representing the entire seasonal cycle, fails to show either mitosis or amitosis taking place in the mesothelium. One would expect frequent division to take place in the mesothelium, considering the great number of cells which must necessarily be derived from it if it be considered as the annual source of replenishment for the depleted lobules.

Figures 10 to 12, all taken from different regions of the same testis, show in the mesothelium features which suggest a possible migration of cells from the surface of the testis. In figures 10 and 11 it would appear that fixation occurred when the cells were in a state of active migration. Figure 10 shows two mesothelial nuclei, one crescentic or bean-shaped and apparently ready to leave the surface epithelium, another much lobulated and depressed from the surface; however, both are still a part of the epithelium. Figure 11 shows an elongated nucleus which might be interpreted as an inward migration. Presumably, this cell has become detached from the mesothelium and is migrating into the stroma. However, it may be a stroma cell which has assumed this position and hence bears no relation to the mesothelium. In figures 10 to 12 the nuclei are much enlarged, and in figure 12 several nuclei appear to be initiating migration, while in the underlying stroma is a mass of irregular-shaped or lobulated nuclei for which an origin from the mesothelium might be postulated. Possibly all of these cells originated from the portion of the epithelium shown in the section or it may be true that they are from neighboring areas and only secondarily collected at this point. On the other hand, it is entirely possible that

the submesothelial cells of this figure bear no genetic relation to the mesothelium, but are an aggregation of stroma cells from a source other than the mesothelium.

Following expulsion of the spermatozoa, the testis is in a semicollapsed state until the lobules become filled with spermatogonia. It is during this time, as previously stated, that the mesothelial nuclei show pronounced changes in size and form. Once the lobules are filled with spermatogonia, the mesothelial nuclei do not show the pronounced enlargement characteristic of the early periods in the reorganization of the testis. If it be true that cells migrate from the mesothelium into the fibrous stroma of the testis, for which positive evidence is lacking, such migrations are always preceded by a definite increase in cell size. The possibility of migration of mesothelial cells without appreciable increase in cell size would be of some importance, for certainly cells smaller than those shown in figures 10 to 12 and capable of transforming or actually transforming into spermatogonia can be observed outside of and within the lobules of figures 4 to 6. Unless migration does occur with little or no previous increase in cell size, it would appear that such cells might originate from other sources, possibly the stroma. In all the material examined, representing the entire seasonal cycle, possible cases of migration without a previous increase in cell size were not observed.

Iron-alum hematoxylin brings out little if any staining difference between the nuclei of the mesothelium of the testis and the nuclei of the stroma (figs. 4 to 12). Positive evidence cannot be furnished for a migration of cells from the testicular mesothelium of *Umbra*. However, even if migration be granted, such cells are indistinguishable from the stroma cells, showing no morphological characteristics or staining reactions whereby one might trace their genetic continuity with the transforming spermatogonia of either the stroma or lobules. While the rôle of the mesothelium in replenishment of germ cells to the annually depleted lobules of teleosts must remain an open question, the writer is of the opinion

that in Umbra the testicular mesothelium does not serve as a source of origin for spermatogonia.

STROMA CELLS

In form, one may discern all manner of possible gradations between the nuclei of stroma cells in the capsule and interlobular trabeculae and the transforming germ cells within the lobules (figs. 4 to 6). As shown in these figures, the nuclei of the stroma may have elongated, oval, rounded, or irregular forms with lobed outlines. In areas of many of the testes examined, during different periods in the spermatogenetic cycle, spermatogonial formation can be observed either in the fibrous tissue or within the lobules, although there is no evidence of even an apparent migration of cells from the testicular epithelium. It may be seen in figures 4 to 6, which show transforming spermatogonia, that the mesothelial nuclei do not exhibit signs of migration. Formation of spermatogonia, under such conditions, is an important point in the favor of stroma cells as the source of germ-cell replenishment. An examination of figure 4 not only demonstrates the origin of spermatogonia from small irregular-shaped cells within the lobule, but also shows, in the stroma, cells transitional with or comparable in size, shape, and staining reaction to those within the confines of the lobule. Cells seen in this figure, intermediate in size between small stroma cells (*C*) and transforming spermatogonia within the confines of the lobule, may have taken origin from remote areas of mesothelium and migrated into this region, but the picture furnished by the preparation indicates that they are growth phases of smaller stroma cells, and no observation has warranted the assumption that these latter arise from the mesothelium. Cells *D* and *E* seem to be actively engaged in migrating into the interior of the lobule. As previously noted, stroma cells other than *D* and *E* of this figure likewise present the possibility of adding to the spermatogonia within either this or more remote lobules, judging from their resemblance in size, shape, and staining reaction either to cells *D* and *E* or to the transform-

ing spermatogonia within the lobule. The evidence seems to justify the conclusion not only that the spermatogonia of figure 4 originated from stroma cells similar to *D* and *E*, but also that the latter were formed as growth phases of cells similar to *C*. The hypothesis that *D* and *E* are the product of growth processes of smaller stroma cells is supported by the presence in the stroma of cells transitional between *C* and *D* or *E*. Figure 5 shows, scattered throughout the stroma and within the lobules, cells which vary in size between stroma cells (*C*) and young spermatogonia (*F*), but comparable in their shape to the latter. The presence in the fibrous tissue of stages intermediate between typical stroma cells (*C*) and transforming spermatogonia (*F*), together with a lack of migratory activity in areas of testis such as represented in figure 5, points, as in figure 4, to a participation of the stroma in the formation of spermatogonia. Figure 6 shows similar possible transitions between stroma cells and spermatogonia. In the latter transformations are taking place in the fibrous tissue of the capsule. It is entirely possible that the spermatogonia of this figure have formed from stroma cells. Cells such as (*C*) in figure 6 evidently have, by processes of growth and differentiation, formed the spermatogonia of this lobule. Lobules such as the one depicted in figure 6 are of frequent occurrence. They are to be found in considerable numbers in many of the testes examined, preceding and following expulsion of the spermatozoa and throughout the period of growth in the testis. Formation of such lobules from transforming cells of the stroma is an added point against the origin of the annual crop of germ cells from reserve cells.

If cells do migrate from the mesothelium into the stroma, for which positive evidence is lacking in Umbra, their identity is lost at their entrance, and in the determination of the history of stroma cells it is impossible, therefore, to establish a differential origin from the mesothelium of those which contribute spermatogonia.

Therefore, in summary, it is concluded that, in *Umbra* at least, spermatogonia arise from transformations of stroma cells of the testis which, in the process, may migrate into previously existing lobules or may transform in situ forming new lobules. This conclusion is based upon the following observations: 1) the testicular mesothelium exhibits neither cell division nor positive evidence of migration either to form spermatogonia or stroma cells; 2) the occurrence in the stroma of cells comparable in size, form, and staining reaction to the transforming spermatogonia within the lobules (figs. 4 and 5) together with the migration of such cells (fig. 4, *D* and *E*) into the lobule; 3) the probable origin of spermatogonia from stroma cells such as (*C*) of figures 4 to 6, which is suggested by the presence in the stroma of cells transitional between such as (*C*) and the early growing spermatogonia within the lobules; 4) the transformation, in the stroma outside existing lobules, of stroma cells into spermatogonia (figs. 3, 6, 8).

LITERATURE CITED

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- HARGITT, G. T. 1924 Germ-cell origin in the adult salamander, *Diemetylus viridescens*. *Jour. Morph. and Physiol.*, vol. 39, no. 1, pp. 63-96.
- TURNER, C. E. 1919 The seasonal cycle in the spermary of the perch. *Jour. Morph.*, vol. 32, no. 3, pp. 681-705.

PLATES

EXPLANATION OF PLATES

All figures were drawn at table level with a camera lucida, using a 1.9 mm. objective and a 10 $\times$ ocular, and are here reduced to one-half their original size. All figures except 10 to 12 were drawn from transverse sections of the testes; figures 10 to 12 represent the sagittal plane.

ABBREVIATIONS

A, cytoplasm of mesothelium; *B*, nucleus of mesothelium

PLATE 1

EXPLANATION OF FIGURES

Only a few spermatozoa, which actually fill the lobules, are shown in figures 1 to 3.

1 Portions of two peripherally placed lobules which have just completed spermiogenesis. It will be noted that there is an absence of cells other than spermatozoa within the lobules.

2 Portion of a centrally located lobule, showing spermatogonia in various stages of development. Presumably, cells similar to *H* of figure 3 gave origin to the spermatogonia of this figure.

3 Portions of two peripherally placed lobules, showing in the stroma a resting spermatogonium and a stroma cell (*H*). Cell *H* of this figure appears to be initiating migration into the lobule. The large resting spermatogonium of the figure apparently originated from a cell similar to *H*, which, instead of migrating into the lobule, underwent spermatogonial formation in the fibrous tissue.

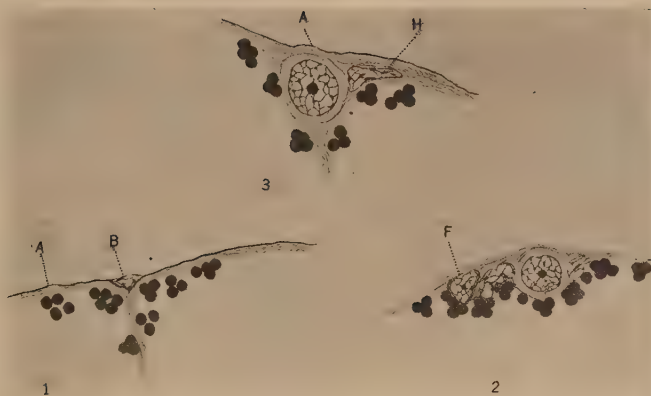


PLATE 2

EXPLANATION OF FIGURES

4 A lobule just before the expulsion of the spermatozoa. Cells *D* and *E* appear to be migrating into the lobule. The spermatogonia lining the inner border of the lobule are in various stages of development, ranging from cells of the migrant type (*E* and *D*) to almost fully formed spermatogonia. Cells similar to *E* and *D* apparently gave origin to the spermatogonia of this figure. In the fibrous tissue are stages intermediate between small stroma cells (*C*) and some of the transforming spermatogonia within the lobule. Many of the cells in the stroma other than *E* and *D* present the possibility of adding to the spermatogonia of either this or more remote lobules. Only a few of the spermatozoa which fill the interior of the lobule are shown in the figure.

5 A view of lobules during the period of reorganization of the testis, illustrating the formation of spermatogonia from cells of the stroma. This figure shows scattered throughout the stroma and within the lobules cells which vary in size between stroma cells (*C*) and young spermatogonia (*F*), but comparable in their shape to the latter.

6 A new lobule forming in the fibrous tissue of the capsule. This figure shows similar possible transitions between stroma cells and spermatogonia to those depicted in figures 4 and 5.

7 A strip of mesothelium and underlying fibrous tissue taken from testis filled with growing spermatogonia. Figures 8 and 9 were taken from different regions of the same testis. It will be noted that the mesothelium is tightly stretched. The mesothelial nucleus shown in this figure is elongated and presents a fairly regular outline.

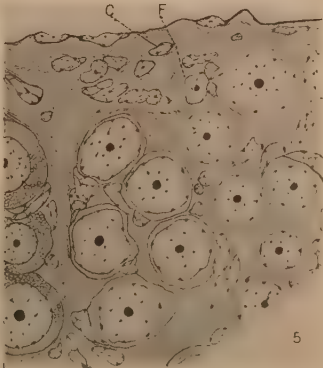
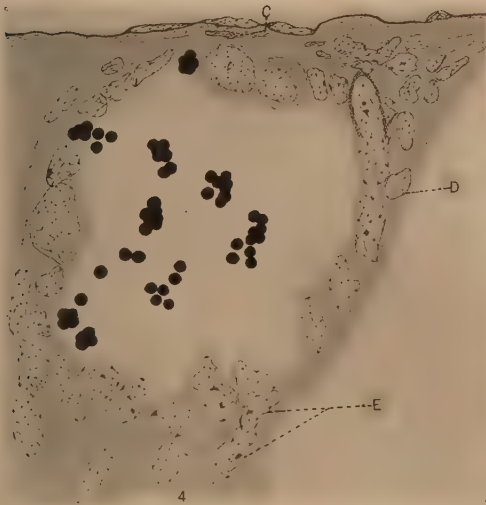
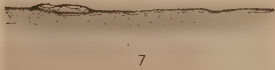
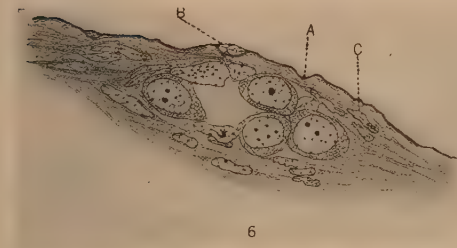
8 A strip of mesothelium and underlying fibrous tissue, showing a spermatogonium transforming in the stroma. It will be observed that the mesothelial nuclei on either side of the spermatogonium show slight extensions into the stroma.

9 A strip of mesothelium and subjacent fibrous tissue taken from an area near the attachment of the swim bladder. Here the contraction of the mesothelium is pronounced. The irregularity in shape of the mesothelial nuclei is to be especially noted.

10 Two mesothelial nuclei are shown in this figure; one crescentic or bean-shaped and apparently ready to leave the mesothelium, another much lobulated and depressed from the surface; however, both are still a part of the mesothelium.

11 This figure shows a nucleus oriented in the stroma with its long axis perpendicular to the mesothelium. This nucleus might be interpreted as an inward migration; however, it may be a stroma cell which has assumed this position, and hence bears no relation to the mesothelium.

12 The mesothelial nuclei of this figure, as of figures 10 and 11, are much enlarged and show extensions into the stroma. Several mesothelial nuclei appear to be initiating migration, while in the underlying stroma is a mass of irregular-shaped or lobulated nuclei for which an origin from the mesothelium might be postulated. However, it is entirely possible that the submesothelial cells of this figure bear no relation to the mesothelium, but are an aggregation of stroma cells from a source other than the epithelium.



BOOKS AND PUBLICATIONS

SYMBIONTICISM AND THE ORIGIN OF SPECIES, by Ivan E. Wallin. 1927. 171 pages, including index and bibliography. Illustrated with plates and graphs. Cloth. Size 6×9 . Price, \$3.00. The Williams & Wilkins Company, Baltimore.

Doctor Wallin holds the conviction that mitochondria are microorganisms symbiotically united with the cell rather than cell organs derived from the cytoplasm. He describes in detail his experiments showing that mitochondria can grow independently of the cell, in artificial culture media. His evidence tends to prove that in many cases new tissues and organs develop as a direct response to microbial invasion. He states that "the fact that mitochondria are universally present in the cells of all organisms higher than bacteria, that the mitochondria are bacterial in nature, and that microsymbiosis can determine morphologic and physiologic changes in organs and cells, can lead to no conclusion other than that symbiontism is a fundamental causative factor in the origin of species." The book summarizes the evidence for the bacterial nature of mitochondria and presents evidence for the rôle played by bacteria in the origin of species. It is a work which may be approved or denounced, but cannot be ignored.

PIERSOL'S NORMAL HISTOLOGY, 13th edition, edited and rewritten by William H. F. Addison. 1927. 477 pages, including index and references. 432 illustrations, 43 of which are in color. Cloth bound. Size $6 \times 9\frac{1}{4}$. Price, \$6.00. J. B. Lippincott Company, Philadelphia.

The author's aim has been to provide by means of minute descriptions of normal tissues a basis for the study of pathological histology. Particular emphasis is laid on those organs most frequently taken at autopsies, and their conspicuous features are discussed. The portions of the book dealing with protoplasm, cell structure, and cell division have been rewritten, the sections on bone-marrow, blood development, and blood cells have been greatly amplified and illustrated with additional figures. The appendix contains descriptions of microdissection, tissue cultures, supravital staining; a discussion of histological technique, special methods, and the injecting of blood vessels.

NUTRITION WORK WITH CHILDREN, by Lydia J. Roberts. 1927. Pages i-xiv, 1-394. Indexed. Illustrated with 14 halftones and 15 charts. Cloth bound. Size $5\frac{1}{2} \times 8\frac{1}{2}$. Price, \$3.50. University of Chicago Press, Chicago.

In this comprehensive survey the author presents the problem of malnutrition and discusses methods for its eradication. It is so well done that the book is bound to appeal to everyone interested in children or in the subject of nutrition. The first part of the volume discusses laws of growth, the nature, causes, and effects of malnutrition and its identification. The latter part outlines methods for combating malnutrition and stresses the necessity for parental education. Welfare workers will find the following chapters particularly valuable: Methods

BOOKS AND PUBLICATIONS

of Judging Nutrition; Studies in Height and Weight; Causes of Malnutrition; Prevention and Treatment of Malnutrition; Nutrition Work with Pre-school children; Parental and Pre-parental Education. Each chapter is followed by an excellent list of references. Miss Roberts not only knows her subject, she knows how to present it.

THE ROCKEFELLER FOUNDATION ANNUAL REPORT FOR 1925. Pages i-xii, 1-521. 81 illustrations. Size $5\frac{1}{4} \times 8\frac{3}{4}$. The Rockefeller Foundation, New York City.

Contains the President's Review; the Reports of the Secretary, the General Director of the International Health Board, the General Director of the China Medical Board, the Director of the Division of Medical Education, the Director of the Division of Studies, and the Treasurer. A comprehensive résumé of the work done by the Foundation during 1925.

ERGEBNISSE DER BIOLOGIE, Zweiter Band. 1927, 729 pages. 177 illustrations. Subject and author indices. Size $6\frac{1}{4} \times 9\frac{1}{2}$. Julius Springer, Berlin.

Das Reizleitungsproblem bei den Pflanzen im Lichte neuerer Erfahrungen, by Peter Stark; Die Blaauwsche Theorie des Phototropismus, by Leo Brauner; Die Georeaktionen der Pflanze, by Walter Zimmermann; Der Harnstoff im Haushalt der Pflanze und seine Beziehung zum Eiweiss, by A. Kiesel; Die Erscheinung der Heteroploidie, besonders im Pflanzenreich, by Fritz von Wettstein; Der Golgische Binnenapparat, Ergebnisse und Probleme, by Werner Jacobs; Histochemie der quergestreiften Muskelfasern, by W. Biedermann; Die Milz, mit besonderer Berücksichtigung des vergleichenden Standpunktes, by Emil v. Skramlik; Die zygotischen sexuellen Zwischenstufen und die Theorie der Geschlechtsbestimmung, by Richard Goldschmidt.

DONNÉES NUMÉRIQUES DE BIOLOGIE ET DE PHYSIOLOGIE ET CHIMIE VÉGÉTALES rédigées par E. F. Terroine et par H. Colin. Extrait du Volume V. Tables Annuelles de Constantes et Données Numériques. 1926. Size $8\frac{1}{2} \times 10\frac{1}{2}$. 123 pages of tables, 15 pages index. Price, unbound, 56 Fr.; bound, 77 Fr. Ch. Marie, Paris.

Collected in one volume are hundreds of tables from articles published in periodicals during the years 1917-1922. The investigator will appreciate the convenience of having these data in compact form rather than scattered inaccessible through numerous journals. The first section of the book is devoted to biology; the second, to botany.

PATHOHISTOLOGIE DER ZÄHNE, mit besonderer Berücksichtigung der Pathobiologie, by Hermann Euler in collaboration with Wilhelm Meyer. 1927. 353 pages. 420 illustrations, 8 of which are in color. Size $7\frac{1}{4} \times 11$. Price, bound, R. M. 49.80; unbound, R. M. 48.00. J. F. Bergmann, München.

This is one of the first books to treat in detail of the histology of diseased human teeth. The author has collected and correlated much new material that has appeared during the last few years in lectures and papers. The illustrations are all actual photographs, insuring perfect accuracy.

BOOKS AND PUBLICATIONS

METHODS AND PROBLEMS OF MEDICAL EDUCATION (Sixth Series). 1927. 271 pages. Illustrated. Size 8×11 . The Rockefeller Foundation, New York.

Twenty-eight articles descriptive of methods and equipment for medical education in fifteen countries. The range of subjects includes libraries, museums, hospitals, clinics, and various departments of medical schools.

X-RAYS AND ELECTRONS, an Outline of Recent X-ray Theory, by Arthur H. Compton. 1926. 418 pages. Illustrated with figures and tables. Cloth. Size $6\frac{1}{2} \times 9\frac{1}{2}$. Price, \$6.00. D. Van Nostrand Company, New York.

To those biologists who are using the x-ray as a tool in the investigation of living material this book will be a welcome addition to the library. It is largely a mathematical exposition and discussion of the theories of x-rays and electrons. Of particular interest are chapters IV and V, which deal with x-ray reflection and crystal structure. The methods therein described are obviously of great potential value in the elucidation of many problems connected with structure in living material.

THE SKATE. A Laboratory Manual, by Charles W. Creaser. 1927. 57 pages, including index. Two illustrations. Cloth. Size $5 \times 7\frac{1}{2}$. The Macmillan Company, New York.

The author states in the preface that the volume is not intended as a treatise on the skate, but rather as an introduction to vertebrate anatomy for inexperienced students. As the skate is a specimen not discussed at length in text-books, it serves as an excellent example for comparison with material on related forms. Mature skates are easy to obtain and are of convenient size and shape for dissection. The manual contains a short chapter of general directions and detailed instructions for the study of external characteristics; the respiratory system; the coelom, digestive system, and mesenteries; the urogenital system; the circulatory system; the nervous system and sense organs; the muscular and skeletal system.

PROTOPLASMA, International Journal of the Physiological Chemistry of Protoplasm. Edited by J. Speck and F. Weber, with the cooperation of R. Chambers and W. Seifriz. Band I, Heft I. July, 1926. 176 pages, including 18 pages of bibliography. Illustrated with text figures and plates. Size $7\frac{1}{2} \times 10$. Price, G. M. 14.00. Gebrüder Borntraeger, Leipzig.

The first issue of a new publication devoted to research of the chemical and physical properties of protoplasm. It contains the following original articles: Protoplasmic Papillae of Echinarachnius Oocytes, by William Seifriz. Cane Sugar and Potassium Nitrate as Plasmolysing Agents, by William A. Beck. Beiträge zur Kenntnis der Plasmolyse, by Ernst Küster. Sur les coefficients de température des différentes phases de la mitose des oeufs d'Oursin, by Boris

BOOKS AND PUBLICATIONS

Ephrussi. There are also a number of book reviews; and a résumé, by Josef Gieklhorn, of the work done on Die Dielektrizitätskonstante (DEK) in der Physiologie.

ANATOMIE ÉLÉMENTAIRE DES CENTRES NERVEUX ET DU SYMPATHIQUE CHEZ L'HOMME, by P. Gilis. 1927. 232 pages, including index. 35 text figures and 1 plate. Size $5\frac{1}{2} \times 8$. Price, 20 francs. Masson et Cie, Paris.

After an introduction of about thirty pages, in which the organogenesis, histogenesis, and cell physiology of the nervous system are briefly considered, the author treats the nervous system as a whole under two divisions, the central nervous system (Système nerveux de la vie de relation) and the sympathetic (Système nerveux de la vie végétative), although he insists at the outset that these two systems are essentially one in function. The central nervous system is treated in two chapters, the first upon the sensory apparatus and the second upon the motor apparatus. The sympathetic system is presented also in two chapters, one upon the sensory apparatus, the other upon the motor and secretory apparatus. A comparatively extended introduction to the second part presents a more general descriptive analysis of the anatomy and physiological characteristics and of the macroscopic divisions of the system: le grand sympathique and le parasympathique. The work is brief, concise, and clear.

THE HARVEY LECTURES. The Twenty-first Series of Lectures delivered under the Auspices of The Harvey Society of New York. 1927. 250 pages. Illustrated. Cloth. Size $5\frac{1}{4} \times 8$. Price, \$4.00. Williams & Wilkins Company, Baltimore.

The volume contains the constitution, lists of officers and members of the Society, and seven lectures making as many contributions to the fields of experimental medicine, physiology, biology, and public health: 1) On Some Recent Otological Problems, by F. R. Nager. 2) The Dynamics of Pepsin and Trypsin, by John H. Northrop. 3) The Transformation of Mononuclear Blood Cells into Macrophages, Epithelioid Cells, and Giant Cells, by Warren H. Lewis. 4) The Parathyroid Glands, by J. B. Collip. 5) Empiricism and Rationalism, by Edwin B. Wilson. 6) Historical Outline of Medical Therapy, by Knud Faber. 7) Comparative Anatomy and Neuropathology, by B. Brouwer.

BOOKS AND PUBLICATIONS

EMINENT CHEMISTS OF OUR TIME, by Benjamin Harrow. Second edition, 1927. 471 pages. Illustrated. Cloth. $5\frac{1}{4} \times 8\frac{1}{4}$. Price, \$3.00. Van Nostrand Company, Inc., New York.

Whether one reads for pleasure or for information, this book is highly satisfactory. The author has written a history of the chemistry of our times by centering it around those men whose work is indissolubly bound up with its progress during the last generation or so. Eleven sketches outline briefly the lives and personalities of some of our outstanding chemists. The second part of the book gives a detailed account of the work of each, with copious references to the original literature. The biologist will find the work of van't Hoff, Arrhenius, and Fischer of particular interest.

COLLEGE ZOOLOGY, revised edition, by Robert W. Hegner. 1926. 645 pages. $6 \times 8\frac{1}{4}$. Index. 553 illustrations. Cloth. The Macmillan Company, New York.

From the preface to the second edition: "This book is arranged so that the student learns where each animal belongs in the animal kingdom; its characteristics and those of the other animals in its class or phylum. One common species is generally used as a type of each large group and then the classification and characteristics of other members of the group are presented more briefly. The groups in the animal kingdom are considered approximately in order of their supposed evolution and thus biological facts and theories fall into a series from the comparatively simple to the more complex in such a way that their gradual evolution is clearly evident. In each group the fundamental biological subjects are studied—thus furnishing the data from which the student arrives at generalization."

BIRTH INJURIES OF THE CENTRAL NERVOUS SYSTEM. Part 1. Cerebral Birth Injuries and Their Results, by Frank R. Ford. Part 2. Obstetrical Injuries to the Spinal Cord, by Bronson Crothers and Marian C. Putnam. With a foreword by Adolf Meyer. 1927. Cloth, gold stamped. 6×9 . 220 pages. 70 illustrations. Tables. Bibliography. Price, \$4.00. The Williams & Wilkins Company, Baltimore.

Dr. Ford presents the first steps of an inquiry into the subject of injuries to the brain at birth, with a survey of the literature and of clinical material. Eliminating congenital paralysis, athetosis, epilepsy, and other diseases frequently attributed to birth trauma, he attempts an exact definition of cerebral birth injuries. Dr. Crothers and Dr. Putnam consider that injuries of the brachial plexus and of the spinal cord are worth investigation from three points of view: 1) They furnish material for the study of physiological disturbances uncomplicated by gross injury of the skeleton or by disease. 2) The problem of treatment and of prognosis demand careful consideration. 3) It is their

BOOKS AND PUBLICATIONS

belief that, almost without exception, injuries of the cord or plexus are due to unphysiological forces imposed upon the foetus. Numerous cases are described and discussed. It is a study of wide appeal to the neurologist, the pediatrician, and the obstetrician.

DIE ANATOMIE DES MENSCHEN, by Friedrich Merkel. Second edition, revised by Erich Kallius. First part: Einleitung, Allgemeine Gewebelehre, Grundzüge der Entwicklungslehre. 1927. 8×11 . 288 pages. Index. 295 illustrations. Price, bound, RM. 23.10; unbound, RM. 21.00. J. F. Bergmann, München.

The first part of the book, a discussion of the individual parts of the cell and the cell as a whole, is followed by a study of the different kinds of tissues—epithelial, connective, muscular, etc. The last chapters are devoted to embryological development. It is equally useful to the zoologist and the student of practical medicine.

AN INTRODUCTION TO THE STUDY OF EXPERIMENTAL MEDICINE, by Claude Bernard. Translated by Henry Copley Greene. Introduction by Lawrence J. Henderson. 1927. 227 pages. $6\frac{1}{4} \times 9\frac{1}{4}$. Cloth. The Macmillan Company, New York.

Unlike the conventional impersonal form generally used in modern scientific papers, the style of this book is vivid and human. Dr. Henderson says in his introduction: "It is not the least of the merits of Claude Bernard's 'An Introduction to the Study of Experimental Medicine' that we have here an honest and successful analysis of himself at work, by one of the most intelligent of modern scientists, a man of genius and a great physiologist." The principal thesis of the work is that medicine has passed through the empirical, the systematic, the nosological, and the morphological stages, and has entered upon the experimental stage; and, as physiology is the larger part of experimental medicine, has become physiological.

ABSTRACTS OF THESES, The University of Chicago Science Series, volume 3. 1927. 351 pages. $9\frac{1}{4} \times 6\frac{1}{4}$. Cloth. Price, \$3.00. University of Chicago Press, Chicago.

The book gives in a compact form a summary of the original research done by students of the Ogden Graduate School of Science. The abstracts represent fifty-five theses submitted to the faculties of the graduate schools for the degree of Doctor of Philosophy, August, 1924, to June, 1925.

THE PLATYPUS. Its Discovery, Zoological Position, Form and Characteristics, Habits, Life History, etc., by Harry Burrell. 1927. 227 pages. 35 plates. Index. $8\frac{1}{4} \times 5\frac{1}{4}$. Cloth. Price, 25 shillings. Angus and Robertson, Ltd., Sydney.

Probably no animal has given rise to more zoological controversy than this peculiar egg-laying mammal. Since the first printed description of the platypus appeared in 1799, there has been so much mystery about its life and habits that Mr. Burrell's authoritative book will be read with greatest interest. The author is well qualified to write about the platypus, having devoted twenty years to its study, both in its natural haunts and in captivity. Every stage of the animal's life history is described from close observation and illustrated with photographs. Its reptilian, avian, and mammalian characteristics are discussed with reference to its position in the animal kingdom.

BOOKS AND PUBLICATIONS

MICROBIOLOGY, by Ward Giltner. Third edition. 1926. Size $5\frac{1}{4} \times 7\frac{1}{2}$. 472 pages, including appendix, index, and bibliography. Illustrated. Cloth. Price, \$3.50. John Wiley & Sons Inc., New York.

This laboratory guide to the study of general microbiology is arranged in three parts. The first part gives a working knowledge of the laboratory methods used in the study of microorganisms. The second consists of exercises demonstrating the various physiological activities of microorganisms. The third deals with applied microbiology. Detailed directions are given for each exercise, so that the student in his work is practically independent. The new edition has been brought up to date and a number of new exercises added, including preparation of aggrassin, the Kahn precipitation test, and a demonstration of bacteriophage.

HANDBUCH DER VERGLEICHENDEN ANATOMIE DER HAUSTIERE, seventeenth edition, by Wilhelm Ellenberger and Hermann Baum. 1926. Size $10\frac{1}{4} \times 8$. 1072 pages. 1373 illustrations. Bibliography. Index. Buckram. Price, R. M. 87. Julius Springer, Berlin.

The introduction treats of the general structure and nomenclature of domestic animals and gives a brief outline of their embryology. The greater part of the book consists of parallel studies of the horse, the cow, the sheep, the pig, the dog, and the cat, comparing them with each other and with man, system by system. Worthy of particular notice are the illustrations of homologous parts showing similarities and differences. Fowls are considered in a special chapter.

VERGLEICHENDE ANATOMIE DER WIRBELTIERE, by J. E. W. Ihle, P. N. van Kampen, H. F. Nierstrasz, and J. Versluys. Translated by G. Chr. Hirsch. 1927. Size 7×10 . 906 pages, including indices. 987 illustrations. Price, unbound, R. M. 66. Bound, R. M. 68.40. Julius Springer, Berlin.

The translation into German of this book, originally published in Dutch, makes more easily available to scientists an excellent work on comparative anatomy. It is made up of a series of separate studies carefully coordinated to produce a complete whole. Each section is written by an authority on that particular subject. Doctor van Kampen discusses the skin, the nervous system, the special sense organs; Doctor Versluys, the skeleton and muscular system; Doctor Ihle, the digestive and urogenital systems, internal organs, and glands; Doctor Nierstrasz, the respiratory and circulatory systems. There are many clear illustrations, a good zoölogical classification, and a complete index.

THE SCIENCE OF BIOLOGY, by George G. Scott. 1925. Size, $5\frac{1}{4} \times 8\frac{1}{2}$. 630 pages. 350 illustrations. Cloth. Price, \$3.50. Thomas Y. Crowell Company, New York.

Lucidity of style and a systematic arrangement of material make a book which even a beginner can read with enjoyment and understanding. It is not merely

BOOKS AND PUBLICATIONS

an absorbing story, however, it is an accurate and comprehensive exposition of the science of biology. It covers a wide field—reaching from protoplasm and the single cell to the biology of man. The introductory section contains definitions and a study of the simplest unit of life. One division treats of plant life, another of animal life. Discussions of general topics and special branches of biological science make up the rest of the volume. Both instructors and students should appreciate a book so interesting and well arranged.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN, Lieferung 231. Edited by Geh. Med.-Rat Prof. Dr. Emil Abderhalden. Abt. IX, Methoden zur Erforschung der Leistungen des tierischen Organismus, Teil 2, 2. Hälfte, Heft 1. Methoden der Süßwasserbiologie.

Hans Helmut Wundsch, Die Arbeitsmethoden der Fischereibiologie, mit 108 Abbildungen, 3 Tafeln und einer lithographischen Tafel.

1927. Size 7×10 . 345 pages. Price M 18.60. Urban und Schwarzenberg, Berlin.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN, Lieferung 232. Edited by Geh. Med.-Rat Prof. Dr. Emil Abderhalden. Abt. IX, Methoden zur Erforschung der Leistungen des tierischen Organismus, Teil 2, 2. Hälfte, Heft 2. Methoden der Süßwasserbiologie.

Sven Ekman, Die Methodik der Tiergeographie des Süßwassers. H. C. Redeke, Flottierende Stationen, mit einer Abbildung. Reinhard Demoll, Adolf Seiser, and Ludwig Walz, Anwendung des Interferometers in der Süßwasserforschung. Carl J. Cori, Die künstliche Anlage von Freilandwasserbecken und Sumpfgärten für Zwecke biologischer und experimenteller Studien und für den Unterrichtsbetrieb, mit 2 Abbildungen. Fritz Lenz, Limnologische Laboratorien, mit 30 Abbildungen. Einar Naumann, Die Lichtverhältnisse des Süßwassers, mit 5 Abbildungen.

1927. Size 7×10 . 176 pages. Price M. 8.70. Urban und Schwarzenberg, Berlin.

HANDBUCH DER MIKROSKOPISCHEN ANATOMIE DES MENSCHEN, bearbeitet von Wilhelm v. Möllendorff. Fünfter Band. Verdauungsapparat. Erster Teil. A. Die Mundhöhle, von S. Schumacher (mit 15 Abbildungen). B. Die Zunge, von S. Schumacher (mit 10 Abbildungen). C. Die Speicheldrüsen der Mundhöhle und die Bauchspeicheldrüse, von K. W. Zimmermann (mit 186 Abbildungen). D. Der lymphatische Rachenring, von T. Hellman (mit 21 Abbildungen). E. Der Schlundkopf, von S. Schumacher (mit 4 Abbildungen). F. Die Speiseröhre, von S. Schumacher (mit 19 Abbildungen). G. Peritoneum einschliesslich Netz, von E. Seifert (mit 21 Abbildungen). Namenverzeichnis, Sachverzeichnis. 1927. Size $7 \times 10\frac{1}{2}$. 374 pages. Price, bound, R. M. 78; unbound, R. M. 72. Julius Springer, Berlin.

METHODS AND PROBLEMS OF MEDICAL EDUCATION (Seventh series). 1927. Size 8×11 . 99 pages. Illustrated. The Rockefeller Foundation, New York.

Descriptions of the buildings, methods of instruction, and experiments in teaching in the various departments of the School of Medicine and Dentistry, University of Rochester, Rochester, New York.

Tenth International Congress of Zoology

BUDAPEST, SEPTEMBER 4 to 9, 1927

At the Ninth International Congress of Zoology, which met at Monaco in March, 1913, it was unanimously resolved to accept the invitation of the Royal Hungarian Ministry of Public Worship and Education to hold the Tenth Congress at Budapest in 1916 under the Presidency of Dr. G. Horváth, Director of the Hungarian National Museum. Owing to the war, it was impossible to carry out this decision, and the Tenth Congress had to be postponed. The international situation has meanwhile so greatly improved that there is no longer any reason for further delay. We have, therefore, the honor to inform you herewith that the Tenth International Congress of Zoology will meet at Budapest from the 4th to the 9th of September, 1927, under the patronage of the International Union of Biological Sciences, and we cordially invite all zoologists and those interested in this science to take part in the meeting.

DR. KUNO COUNT KLEBELSBERG, *Privy Councillor*
Hungarian Minister of Public Worship and Education,
representing the Royal Hungarian Government

DR. FRANCIS RIPKA
First Burgomaster of Budapest

DR. ALBERT BERZEVICZY, *Privy Councillor*
President of the Hungarian Academy of Sciences

DR. EUGENE SIPOCZ
Burgomaster of Budapest

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PROF. LOUIS JOUBIN (Paris)
Member of the Institut de France
General Secretary of the Ninth International
Congress of Zoology, representing the Permanent
Committee of the International Congresses of
Zoology.

DR. G. HORVÁTH (Budapest)
Member of the Hungarian Academy of Sciences
Former Director, Hungarian National Museum.
*President of the Tenth International
Congress of Zoology*

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	Dr. M. Pekár (Pécs)	D. Zilahy (Budapest)

Members of the Hungarian Organizing Committee

PRELIMINARY PROGRAM

The program has provisionally been arranged as below. A final and more detailed program will be issued in good time before the Congress. All who wish to take part in the Congress are requested to fill in the forms sent them and to return them as soon as possible to the President of the Congress, Dr. G. Horváth (Hungarian National Museum, Budapest, 80), who will be pleased to give any further information required. The Organizing Committee in Hungary will do everything in its power to arrange for facilities in connection with the journey and the sojourn in Hungary (50 per cent reduction on the Hungarian passport-visa, reduced prices on the railways and in hotels, accommodation in University Colleges, etc.).

Those taking part in the Congress will be either members or associates.

Members paying a fee of 25 pengó (= 18 sh. = \$4.50) are qualified to vote at all meetings of the Congress, to deliver one or more lectures, to bring proposals before the meetings, and to take part in the discussions, receptions, excursions, etc.; they will also receive one copy of the Transactions of the Congress.

Associates paying a fee of 12.50 pengó (= 9 sh. = \$2.25) are entitled to be present at all functions of the Congress, but have no right to vote at the meetings, or to lecture, or to take part in the discussions, nor will they receive the Transactions.

Members and Associates are requested to send their fees by cheque to the Hungarian Commercial Bank of Pest, Branch Belváros (Budapest, IV., Károly-körút 1.) payable to the account of the Tenth International Congress of Zoology. The membership card will be sent as a receipt.

The offices of the Congress will be in the Hungarian National Museum.

The formal opening ceremony as well as the farewell meeting and the general meetings will be held in the large hall of the Hungarian National Museum, whereas the sectional meetings will take place in the rooms of the University institutes for natural history and for medicine.

There will be four general meetings and a number of sections, the latter being provisionally arranged as follows:

1. General zoology.
2. Experimental cytology (tissue culture).
3. Vertebrata (morphology, physiology, evolution, systematics).
4. Invertebrata (morphology, physiology, evolution, systematics) exclusive of Arthropoda.
5. Arthropoda (morphology, physiology, evolution, systematics).
6. Applied zoology (animals useful or injurious).
7. Palaeozoology.
8. Zoogeography.
9. Nomenclature.

According to the number of papers announced, each section may be subdivided or several sections may be united.

MEETINGS

Saturday, September 3rd, 8 P.M., the members and associates will be received and entertained by the municipal authorities of Budapest at the Hotel St. Gellért.

Sunday, September 4th

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| <p>9 A.M. Meeting of the Permanent Committee of the International Congresses of Zoölogy to select for election the vice-presidents, the general secretary of the Tenth Congress, the presidents of the sections, and new members of the Permanent Committee.</p> <p>4 P.M. Steamer excursion on the Danube.</p> | <p>11 A.M. Formal opening of the Congress. Addresses. Election of officers and of new members of the Permanent Committee. Introduction of delegates. Organization of the sections. Two lectures of general interest.</p> <p>6 P.M. Landing on St. Margaret's Island.</p> |
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Monday, September 5th

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| <p>9 A.M. First general meeting.</p> <p>3 P.M. Sections.</p> <p>3 P.M. Meeting of the International Commission on Zoölogical Nomenclature.</p> | <p>7 P.M. Lecture by Dr. Eug. Cholnoky, professor, on "Lake Balaton and the Puszta Hortobágy in Hungary (with lantern slides)."</p> |
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Tuesday, September 6th

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| <p>9 A.M. Second general meeting.</p> <p>3 P.M. Sections.</p> | <p>3 P.M. Meeting of the International Commission on the Concilium Bibliographicum.</p> |
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Wednesday, September 7th

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| <p>9 A.M. Third general meeting.</p> <p>3 P.M. a) Visit to the Royal Hungarian Institute of Geology (palaeontological collections), to the Royal Hungarian Museum of Agriculture, and to the Zoölogical Gardens.</p> | <p>b) Excursion to the Svábhegy (Swabian Mountain, 485 meters) and the Jánoshegy (St. John's Mountain, 529 meters) in the neighborhood of Budapest.</p> |
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Thursday, September 8th

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| <p>9 A.M. Fourth general meeting.</p> <p>3 P.M. Sections.</p> <p>3 P.M. Meeting of the International Commission on Parasitology.</p> | <p>8 P.M. Banquet given at the Grand Hotel Hungaria by the Tenth International Congress of Zoölogy to its members and associates.</p> |
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Friday, September 9th

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| <p>9 A.M. Sections.</p> <p>11 A.M. Meeting of the Permanent Committee of the International Congresses of Zoölogy to consider the place and President of the Eleventh Congress in 1930.</p> | <p>4 P.M. Formal farewell meeting. Report of the General Secretary of the Congress. Selection of the place and election of the President of the Eleventh Congress. Various proposals of general interest. Farewell address of the President.</p> |
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EXCURSIONS

Arrangements will be made for two excursions to take place after the Congress, one to Lake Balaton and the other to the Puszta Hortobágy.

Saturday, September 10th

Excursion to Lake Balaton and official inauguration of the new building of the Hydrobiological Station of Tihany. (Departure from Budapest in the morning, return in the evening.)

Sunday and Monday, September 11th and 12th

Visit to the Puszta Hortobágy with its herds of horses, cattle, and sheep; inspection of the installation of Pisciculture. (Leave Budapest on September 11th during afternoon for Debrecen, where the night will be spent; next morning by train to the Puszta Hortobágy, where luncheon will be provided; in the afternoon, return to Budapest, arriving in the evening.)

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